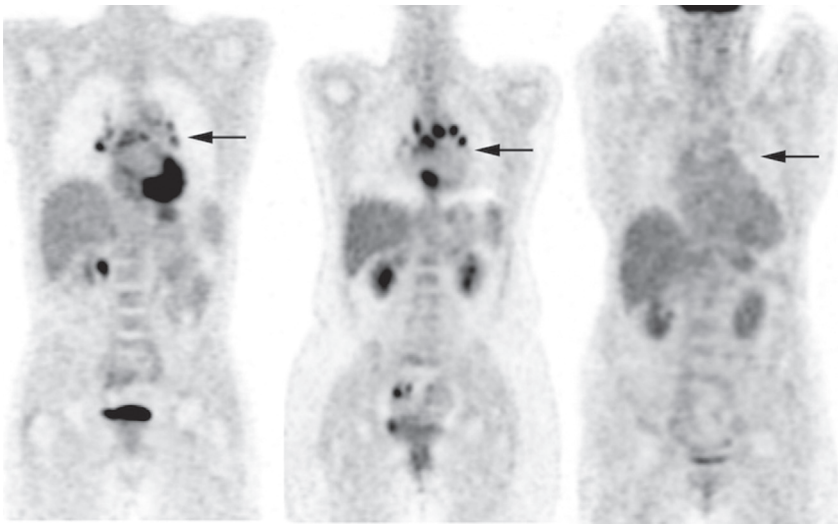
DISSERTATIONES SCHOLAE DOCTORALIS AD SANITATEM INVESTIGANDAM
UNIVERSITATIS HELSINKIENSIS

104/2015

RIINA KANDOLIN

Cardiac Sarcoidosis and Giant Cell Myocarditis in Finland



HELSINKI UNIVERSITY HOSPITAL
DIVISION OF CARDIOLOGY
HEART AND LUNG CENTER
FACULTY OF MEDICINE
DOCTORAL PROGRAMME IN CLINICAL RESEARCH
UNIVERSITY OF HELSINKI

Division of Cardiology, Heart and Lung center

Helsinki University Hospital

Helsinki, Finland

and

Faculty of Medicine, University of Helsinki

Helsinki, Finland

Cardiac Sarcoidosis and Giant Cell Myocarditis in Finland

Riina Kandolin

ACADEMIC DISSERTATION

To be presented, with the permission of The Faculty of Medicine of the University of Helsinki, for public examination in Auditorium 2, Meilahti Hospital,
on December 11th 2015, at 12 noon.
Helsinki 2015

Supervisors

Docent Jukka Lehtonen, M.D., Ph.D.
Heart and Lung Center, Helsinki University Hospital
and University of Helsinki
Helsinki, Finland

Professor Markku Kupari, M.D., Ph.D.
Heart and Lung Center, Helsinki University Hospital
and University of Helsinki
Helsinki, Finland

Reviewers

Docent Ville Kytö, M.D., Ph.D.
Heart Center, Turku University Hospital
Research Centre of Applied and Preventive Cardiovascular
Medicine, University of Turku
Turku, Finland

Docent Tuomas Rissanen, M.D., Ph.D.
Central Hospital of North Karelia, Joensuu and University of
Eastern Finland,
Kuopio, Finland

Opponent

Assistant Professor, Antti Saraste, M.D., Ph.D.
Heart Center, Turku University Hospital
Turku, Finland

ISBN 978-951-51-1768-7 (Paperback)
ISBN 978-951-51-1769-4 (PDF)
ISSN 2342-3161 (Print)
ISSN 2342-317X (Online)

Hansaprint, Finland 2015

"Live as if you were to die tomorrow. Learn as if you were to live forever".

Mahatma Gandhi (1869-1948)

To Johannes and Adrian

Table of Contents

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Kandolin R, Lehtonen J, Airaksinen J, Vihinen T, Miettinen H, Ylitalo K, Kaikkonen K, Tuohinen S, Haataja P, Kerola T, Kokkonen J, Pelkonen M, Pietilä-Effati P, Utriainen S, Kupari M. Cardiac Sarcoidosis: Epidemiology, Characteristics, and Outcome over 25 Years in a Nationwide Study. *Circulation.* 2015; 131: 624-632.

II Kandolin R, Lehtonen J, Graner M, Schildt J, Salmenkivi K, Kivistö SM, Kupari M. Diagnosing isolated cardiac sarcoidosis. *J Intern Med.* 2011; 270: 461–468.

III Kandolin R, Lehtonen J, Kupari M. Cardiac Sarcoidosis and Giant Cell Myocarditis as Causes of Atrioventricular Block in Young and Middle-Aged Adults. *Circ Arrhythm Electrophysiol.* 2011; 4: 303-309.

IV Kandolin R, Lehtonen J, Airaksinen J, Vihinen T, Miettinen H, Kaikkonen K, Haataja P, Kerola T, Kupari M. Usefulness of Cardiac Troponins as Markers of Early Treatment Response in Cardiac Sarcoidosis. *Am J Cardiol*. 2015; 116: 960-964.

V Kandolin R, Lehtonen J, Salmenkivi K, Räisänen-Sokolowski A, Lommi J, Kupari M. Diagnosis, Treatment, and Outcome of Giant Cell Myocarditis in the Era of Combined Immunosuppression. *Circ Heart Failure*. 2013; 6: 15-22.

 In addition this thesis includes some previously unpublished data.

ABBREVIATIONS

ARVC	Arrhythmogenic right ventricular cardiomyopathy
AV-block	Atrioventricular block
CRT	Cardiac resynchronization therapy
CS	Cardiac sarcoidosis
DCM	Dilated cardiomyopathy
ECG	Electrocardiogram
18-FDG-PET	18-fluorodeoxyglucose positron emission tomography
GCM	Giant cell myocarditis
Hs-cTnI	High-sensitivity cardiac troponin I
Hs-cTnT	High sensitivity cardiac troponinT
HLA	Human leucocyte antigen
HUH	Helsinki University Hospital
ICD	Intracardiac cardioverter defibrillator
JMHW	Japanese Ministry of Health and Welfare
LGE-CMR	Late gadolinium enhancement cardiac magnetic resonance imaging
LBBB	Left bundle branch block
LV	Left ventricle
LVEDD	Left ventricle end-diastolic diameter
LVEF	Left ventricle ejection fraction
NYHA Class	New York Heart Association functional class
NT-proBNP	N-terminal pro-B-type natriuretic propeptide
RBBB	Right bundle branch block
RV	Right ventricle
Th-1	T-helper-1 cell
Th-2	T-helper-2 cell
TNF-α	Tumor necrosis factor alpha
VF	Ventricular fibrillation
VPB	Ventricular premature beat
VT	Ventricular tachycardia

ABSTRACT

The goal of this study was to assess the epidemiology, characteristics and outcome of cardiac sarcoidosis (CS) and giant cell myocarditis (GCM) in Finland.

CS and GCM are underdiagnosed inflammatory myocardial diseases. Sarcoidosis is a systemic disease characterized by granuloma formation and subsequent tissue scarring in various organs, most commonly in the lungs. In majority of cases, lung sarcoidosis is a self-limiting disease whereas cardiac involvement carries a poorer prognosis due to heart failure and malignant arrhythmias. GCM is a rare, frequently fatal myocardial disease designated by widespread myocardial inflammation and necrosis. In this study we identified and analyzed all cases with histologically confirmed CS and GCM in Finland between 1988 and 2014.

A marked increase in the detection rate of CS in Finland over the last 26 years was found. In the era of modern diagnostic imaging and increased awareness, the annual detection rate of CS is over 50 times higher than before. Anyhow, CS is still a rare disease with prevalence of 2.2/100 000 of histologically verified cases in 2012. Altogether 110 biopsy-confirmed CS cases diagnosed between 1988 and 2012 form the core of this study.

CS most commonly manifests with atrioventricular block (AV-block). What is more, CS and GCM together explain 25% of initially idiopathic 2nd to 3rd degree AV-blocks in adults aged 18-55 years. Other principal manifestations of CS are heart failure and ventricular arrhythmias. Two thirds of patients with CS present without prior diagnosis of sarcoidosis, an entity called clinically isolated CS. The mean age of CS patients was 51 years and two thirds were female. The diagnosis was based on endomyocardial biopsy (EMB) in 50% of cases and on extracardiac biopsy combined with cardiac imaging findings (cardiac magnetic resonance (CMR) or positron emission tomography (PET)) in 50% cases. Single EMB session had a sensitivity of approximately 30% in detecting CS, but repeated biopsies or taking histologic samples from mediastinal lymph nodes markedly improved the diagnostic yield. High-sensitivity cardiac troponins T/I were frequently elevated in treatment-naïve CS, but decreased consistently after initiation of corticosteroid treatment.

In majority of cases, CS is a slowly progressive cardiomyopathy. Over the median follow-up of 7 years, 32 of 110 patients suffered a cardiac death or aborted sudden death or underwent cardiac transplantation. With up-to-date diagnostic methods and treatment including immunosuppression and ICDs, 99% of patients were alive without cardiac death or transplantation at 1 year and 91% after 10 years from symptom onset. Had the systolic heart failure already developed by the time of diagnosis, the prognosis was worse.

Altogether 32 biopsy-verified GCM cases were identified with increasing detection rate over a 20-year span in Finland. Twenty-six of 32 cases were diagnosed by myocardial biopsy. The most common presentations in GCM patients were heart failure and AV-block. Moreover, ventricular arrhythmias were common with two third of patients experiencing sustained ventricular tachycardia or ventricular fibrillation during the disease course. Fifteen of 32 GCM patients either died or underwent cardiac transplantation a median 11 months from symptom onset. With current diagnostic methods and therapy with a combination of immunosuppressants, the transplant free survival was 69% at 1 year and 52% at 5 years.

In conclusion, the detection rate of CS and GCM in Finland is increasing and the prognosis with contemporary diagnostic and therapeutic methods seems better than previously reported.

TIIVISTELMÄ

Tutkimuksen tavoitteena oli selvittää sydänsarkoidoosin ja jättisolumyokardiitin (JSM) taudinkulkua sekä niiden yleisyyttä Suomessa.

Sydänsarkoidoosi ja JSM ovat alidiagnosoituja sydänlihaksen tulehduksellisia sairauksia. Sarkoidoosi on monielinsairaus, jossa tyypillisimmin keuhkoihin muodostuu granulomatoottisia tulehdussolukertymiä. Keuhkosarkoidoosin ennuste on yleensä hyvä, sitä vastoin granuloomien ilmaantuminen sydämeen voi johtaa kammioperäisiin rytmihäiriöihin ja sydämen vajaatoimintaan. JSM on usein nopeasti etenevä sairaus, jossa autoimmuunimekanismilla syntynyt tulehdus aiheuttaa sydänlihaksen nekroosia ja fibrotosoitumista. Tässä tutkimuksessa keräsin ja analysoin yhdessä kansallisen tutkijaryhmän kanssa Suomessa vuosina 1988-2014 todetut sydänsarkoidoosi ja JSM tapaukset.

Yksi tutkimuksen päätuloksista oli se, että diagnosoitujen sydänsarkoidoositapausten määrä on voimakkaassa kasvussa. Tutkimusjakson aikana vuosittain diagnosoidun sydänsarkoidoosin ilmaantuvuus kasvoi yli 50 kertaiseksi, johtuen ensisijaisesti sydämen kuvantamismenetelmien kehityksestä ja kliinikoiden lisääntyneestä tietoisuudesta. Sydänsarkoidoosin esiintyvyys Suomessa vuonna 2012 oli 2.2/100 000 asukasta. Vuosien 1988 ja 2012 välillä todettiin 110 sydänsarkoidoosi tapausta ja tämä tutkimus perustuu näiden potilaiden analyysiin.

Sydämen sähköinen johtumishäiriö on sydänsarkoidoosin tavallisin ensioire. Sydänsarkoidoosi ja JSM yhdessä selittävät neljänneksen 18-55-vuotiaiden aikuisten etiologialtaan alun perin tuntemattomista II-III asteen eteiskammiokatkosista. Muita sydänsarkoidoosin ilmenemiä ovat kammioperäiset rytmihäiriöt ja sydämen

vajaatoiminta. Aineistossamme 2/3:lla oli sarkoidoosin aiheuttamia oireita ainoastaan sydämessä. Sydänsarkoidoosi potilaiden keski-ikä oli 51 vuotta ja heistä 2/3 oli naisia. Sydänsarkoidoosi diagnosoidaan joko sydänlihaskoepalasta tai kun muusta kudoksesta todetaan sarkoidoosi ja sydämen magneettikuvaus tai positroniemissiotomografia sopivat sydänsarkoidoosiin. Yksittäisen sydänlihasbiopsian herkkyys sydänsarkoidoosin havaitsemisessa on noin 30% johtuen mikroskooppisten granuloomien läiskittäisestä jakautumisesta sydänlihaksessa. Biopsioiden kohdentaminen, toistetut sydänlihasbiopsiat ja näytteenotto välikarsinan imusolmukkeista parantavat huomattavasti diagnostiikan osuvuutta. Diagnoosivaiheessa seerumin herkkä troponiini T/I oli koholla yli puolella sydänsarkoidoosi potilaista, mutta laski johdonmukaisesti kortikosteroidihoidon aloituksen myötä.

Yleensä sydänsarkoidoosi on hitaasti etenevä sairaus. Seitsemän vuoden keskimääräisen seuranta-ajan kuluessa 32 potilasta 110:stä menehtyi sydänperäisesti, sai sydämensiirron tai selvisi äkkikuolemasta rytmihäiriötahdistimen tai tehokkaan elvytyksen ansiosta. Nykyisen immunosuppressiohoidon ja rytmihäiriötahdistimien aikakaudella vuoden kuluttua oireiden alusta oli elossa ilman sydämensiirtoa 99% ja 10 vuoden kuluttua 91% potilaista. Sydämen vajaatoimintaan ensioireena liittyi huonompi ennuste.

Kahdenkymmenen vuoden kuluessa Suomessa todettiin 32 koepalalla varmistettua JSM tapausta ja myös JSM:a todettiin enenevässä määrin seuranta-aikana. Eteiskammiojohtumisen häiriöt ja sydämen vajaatoiminta olivat yhtä yleisiä ensioireita JSM:ssa. Kammioperäisiä rytmihäiriöitä ilmeni seurannassa valtaosalla. Kymmenen potilasta sai sydämensiirron ja 5 menehtyi keskimäärin 11 kuukauden kuluttua oireiden alusta. Nykyisten tunnistusmenetelmien ja yhdistelmä immunosuppressiohoidon aikana kaikista JSM potilaista vuoden kuluttua oireiden alusta oli elossa ilman sydämensiirtoa 69% ja 5 vuoden kuluttua 52%.

Yhteenvetona todetaan, että sydänsarkoidoosin ja JSM:n määrä Suomessa on kasvussa ja niiden ennuste nykyisten tutkimus- ja hoitomenetelmien aikana näyttää aiempaa suotuisammalta.

1 INTRODUCTION

Sarcoidosis with cardiac involvement was first recognized almost 100 years ago but still it remains underdiagnosed (1). Cardiac sarcoidosis (CS) is detected in around 5%, yet present in approximately 20-30% of sarcoidosis patients. In cardiology practice, CS presents as atrioventricular block (AV-block), ventricular arrhythmias and systolic or diastolic heart failure. The most feared outcome is sudden cardiac death. The diagnosis of CS is difficult since no singular laboratory or imaging finding can confirm it. The diagnostic golden standard is endomyocardial biopsy (EMB) revealing non-caseating granulomas. However, due to the uneven myocardial distribution of granulomas a single, untargeted EMB procedure more often misses than hits them (2,3). Additionally, CS diagnosis can be made based on extracardiac histologic confirmation of sarcoidosis, combined with clinical presentation of a myocardial disease together with compatible imaging findings in echocardiograhy and/or late gadolinium enhancement cardiac magnetic resonance imaging (LGE-CMR) and/or 18-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) (4). The outcome data on CS is heterogeneous depending on the type of study (autopsy/lifetime) and diagnostic criteria used. In the largest lifetime studies, the five-year survival was 60-90% (4-6).

The first case report of giant cell myocarditis (GCM) was published over 100 years ago. However, due to the rarity and often rapidly progressive nature of the disease, there is only limited data on GCM. Autoimmune responses, infections and genetic predisposition have been implicated in its pathogenesis (7). GCM often presents with fulminant heart failure and resistant ventricular arrhythmias, but more indolent disease course has also been described (5,8,9). A definite diagnosis of GCM is based on EMB showing multiple giant cells, eosinophils and myocardial necrosis (10). The reported prognosis of GCM is grim with a rate of death or transplantation of 89% with a median of 5.5 months from symptom onset in a GCM landmark study (11). Despite this, over one third of patients were diagnosed only after autopsy or cardiac transplantation and in patients with pre-mortem and pre-transplant diagnosis receiving combined immunosuppression, the median survival time was 12.3 months.

There is no prior data concerning the epidemiology of CS and GCM in Finland and worldwide the data is scarce. The effect of novel diagnostic methods on the diagnostics of CS and GCM is unclear. Furthermore, the disease course and outcome with contemporary treatment including immunosuppression and intracardiac cardioverter defibrillators (ICDs) are uncertain. I set out to study these questions.

2 REVIEW OF THE LITERATURE

2.1 Historical perspective

The written story of sarcoidosis saw daylight in 1869 when the British dermatologist-surgeon Jonathan Hutchinson first described a case of cutaneous lesions, different from tuberculosis, syphilis, lupus and other theretofore known skin diseases, at King`s College, London (12). The illness was named and histologically defined by the Norwegian Caesar Boeck who examined skin biopsies in 1899. The naming was purely descriptive as Boeck found that the epitheloid cells in granulomas resembled sarcoma cells and coined the term ”sarcoid” (13). In 1914, the Swede Jorgen Schaumann synthesized previous heterogeneous case reports of multiple organ manifestations into a single disorder and called it ”lymphogranulomatosis benigna” to distinguish it from malignant lymphoma (12,14).

Cardiac involvement in a patient with systemic sarcoidosis was first documented by Bernstein in 1929 (13). In 1952, Longcope and Freiman found myocardial sarcoid involvement in a total of 20% of autopsy cases and in the literature of that time (15). For decades, CS was diagnosed mainly post-mortem, often after sudden cardiac death, and only a minority (12%) of cases were clinically diagnosed (16). Two widely cited autopsy studies from the 1970s showed that sarcoidosis commonly affects only small portions of the myocardium and is often clinically silent (16,17). In the 1970s and 80s, Dr. Fleming from Cambridge collected 250 CS cases from the UK (18-20). Prior to the invention of endomyocardial biopsy (EMB), CS in a living person could only be diagnosed on clinical grounds (21). Multiplicity of therapeutic agents has been experimented on sarcoidosis since it was first discovered (12) and by the 1970s and 80s, steroids were already commonly in use in CS (22).

The first case report of GCM was published in 1905, describing a fatal case of myocarditis with unknown origin, histologically characterized by widespread myocyte necrosis and the formation of multinucleated giant cells (23,24). Until the 1950s and 60s the terms “GCM” and “granulomatous myocarditis” or CS were used interchangeably, but since then GCM and CS have been recognized as distinct diseases (25,26). Knowledge about GCM was long based on various case reports (27) and today still consists of rather small case series as the explicitly largest multicenter trial included 73 patients (5).

As the diagnostic methods, EMB in particular, evolved in the 1980s, CS and to a lesser extent GCM gradually started to be recognized pre-mortem. With the development of sensitive imaging methods, advances in electrophysiology and genomic studies in recent years, enthusiasm around CS has risen and the number of publications has increased. Still today, these heterogenic myocardial diseases challenge the treating

physicians as there is no consensus on the optimal diagnostic conduct and management (28).

2.2 Pathogenesis

Sarcoidosis is a systemic immune-mediated disease, characterized by the formation of inflammatory granulomas in various organs. The exact cause of sarcoidosis still remains to be solved (29). The data on pathogenetic mechanisms comes mainly from pulmonary studies, since the lungs are by far the most commonly affected organ (30,31). Extrapulmonary disease is observed in 30-50% patients with lymph nodes, eyes, skin, heart, nervous, musculoskeletal, renal, and endocrine systems being the most commonly affected (13,29,30). Large differences in organ involvement are found between different ethnic groups and parts of the world (30).

The pathologic hallmark of sarcoidosis is a non-necrotizing granuloma, i.e., a nodus containing inflammatory cells (3), **Figure 1**. Macrophages form the central core and are surrounded by T-lymphocytes, plasma cells and mast cells. In mature granulomas this ball-like cell cluster is circulated by fibroblast and collagen ring (32,33). As a result of chronic cytokine stimulation, macrophages differentiate into epithelioid cells, lose some phagocytic capacity, gain secretory and bactericidal properties and fuse to form multinucleated giant cells (33). Finally, in some cases the granulomas become sclerosed. The granulomas are thought to form in order to segregate and prevent non-degradable remnants of the causative agents from spreading to the surroundings but concurrently they distort the tissue architecture, leading to loss of function.

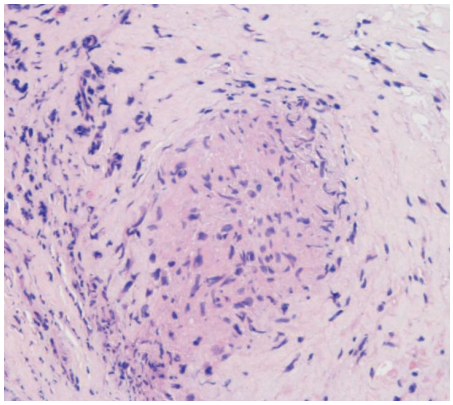


Figure 1. Light microscopic image of a granuloma in CS. (With permission from Dr. Kaisa Salmenkivi, Department of Pathology, HUSLAB, Helsinki University Hospital)

The emerging consensus is that sarcoidosis represents a final common pathway and results from exposure to one or more potential antigens in a genetically predisposed individual (34,35). Yet, there is an ongoing debate concerning the etiology, and even the existence of sarcoidosis as an autonomous disease has been questioned, generating a concept of sarcoid-like reactions to diverse immunologic agents (36). Sarcoidosis

cannot be classified as a classic autoimmune disease, but autoimmunity certainly plays an important role in the pathogenesis. Other autoimmune disorders, most commonly thyroid, thymus and inflammatory bowel diseases, have been reported in approximately 20% of sarcoidosis as well as in GCM patients supporting the autoimmune background 1(11,37,38).

2.2.1 Immunomechanisms

Immunologically, sarcoidosis is considered an exaggerated immune response, in which so far unidentified antigens induce a T-cell mediated immune response (29), **Figure 2**. The T-helper-1 (Th-1) response shifts the cytokine profile towards delayed hypersensitivity, macrophage activation, and pro-inflammatory T-cell response, whereas the T-helper-2 (Th-2) response is associated with B-cell activation and humoral immunity (39). In sarcoidosis, environmental and/or infectious antigens generate a Th-1 lymphocyte immune response, in which Th-1 cells produce T-cell cytokines and oligoclonal proliferation of T-cells, ultimately resulting in granulomas (32,40). Several studies demonstrate that the T-cell response in sarcoidosis-affected tissue is strongly polarized towards a Th-1 activation with low levels of Th-2 activity (41,42). The expression of interferon gamma (IFN-γ, the signature cytokine produced by Th-1 cells), interleukin 2 (IL-2), interleukin 12 (IL-12), tumor necrosis factor alpha (TNF-α), and other cytokines consistent with Th-1 phenotype is upregulated at sites of sarcoid lesions (32,41,42). Finally, as a result of ongoing inflammation and tissue injury in combination with wound healing properties, fibrosis leading to organ dysfunction may be generated (43). The mechanisms leading to fibrosis or resolution are poorly understood and elucidating them would be important in designing new therapies for sarcoidosis (44). In general, the inflammatory cells are localized in affected organs in sarcoidosis patients, while cytokine profile and peripheral blood lymphocyte activation are similar to healthy controls (40,45). Anyhow, recently novel gene expression analyses have been able to identify immunologic differences in peripheral blood (46).

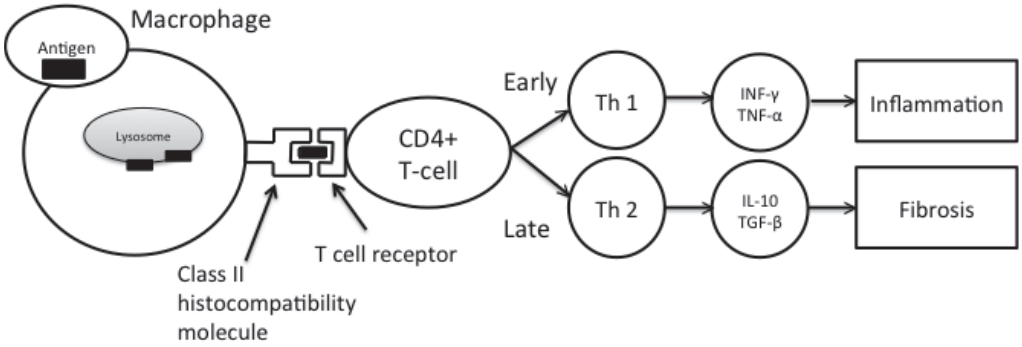


Figure 2. Current view on pathogenesis of sarcoidosis. Adapted from (13).

Th 1= T-helper-1 cell, Th 2= T-helper-2 cell, IFN-γ= interferon gamma, TNF-α= tumor necrosis factor alpha , IL-10= interleukin 10, TGF-β= transforming growth factor β.

2.2.2 Genetic factors

As the immunological mechanisms described above depend on genetic factors, genes play an important role both in the risk of disease as well as organ involvement (29). Genetic predisposition to sarcoidosis has been shown epidemiologically by demonstrating an 80-fold increased risk in monozygotic twins of affected individuals (47) and familial clustering with an almost five times higher risk of sarcoidosis in relatives of sarcoidosis patients (48). Moreover, ethnic variation in disease susceptibility and outcome (30,49) supports genetic influence. Finally, different human leucocyte antigen (HLA) class II (DRB1 and DQB1) genotypes are associated with different sarcoidosis phenotypes and they manifest differently, depending on ethnic background (50).

Candidate gene and genome-wide association studies have identified multiple genes behind the genetic susceptibility to sarcoidosis (46). However, the reported results have often been conflicting, probably due to the heterogenity of the disease entity (51). To date, the strongest genetic associations in sarcoidosis involve HLA class II genes: some are risk factors and others appear protective (34,51). Further, genome-wide association studies have identified various non-HLA genes, including butyrophilin-like 2 protein involved in T-lymphocyte activation, annexin a11 involved in cell apoptosis, and TNF-α genes, associated with sarcoidosis (51). A single nucleotide polymorphism in the toll-like receptor 3 with an essential role in Th1/2 lymphocyte polarization has been associated with susceptibility to CS in Japanese patients with systemic sarcoidosis (52). Recently, epigenetic mechanisms, such as altered microRNA expression, aberrant DNA methylation status, and dysregulation of histone modifications have been demonstrated to affect the transcription activity of genes involved in the immune system and thus act as a mediator between environmental and genetic factors in immune-mediated

pulmonary diseases, including sarcoidosis (53). Furthermore, these gene expression differences offer possible markers for future sarcoidosis diagnostics, which today suffer from lack of sensitivity (54).

2.2.3 Infectious and environmental factors

The immunologic cascade eventually leading to granuloma formation is triggered by an antigenic stimulus. Several potential antigens have been proposed, with the infectious hypothesis being the strongest theory. *Mycobacteria* and *propionibacteria* are common, commensal acid-fast bacilli that can persist in macrophage phagosomes. Killed or partly degraded bacteria are suspected to form a nidus for granuloma formation. Several pieces of evidence support their role. First, bacterial cultures and polymerase chain reaction assays evidencing microbial DNA have identified these organisms at higher percentages in tissues of sarcoidosis patients than in controls (55). Moreover, in experimental animals, sensitization with *propionibacterium acnes* led to granulomatosis, and the eradication of *P. Acnes* by antibiotics prevented the development of granulomas (56).

In addition to infectious agents, several environmental and occupational agents have been entertained in the etiology of sarcoidosis. The multicenter ACCESS (A Case Control Etiologic Study of Sarcoidosis) trial recruited over 700 subjects and was unable to find a single ”cause” of sarcoidosis, but identified several environmental factors, including work environments with mold/mildew causing possible exposure to microbial bioaerosols and occupational exposure to insecticides, with modest association to the risk of sarcoidosis (57). Similarly, studies have reported occupational clustering of sarcoidosis or sarcoid-like reactions in work sites with exposure to inorganic dust, metal dust, silicates, mold and microbially rich air (58). As one example, exposure to the heavy inorganic dust burden following the destruction of the World Trade Center in 2001 was followed by an increased disease rate of 229 per 100,000 in rescue personnel, with 8% cardiac involvement (59).

2.2.4 GCM

Less is known about the pathogenesis and immunomechanisms of GCM. Histologically GCM involves multinucleated giant cells, necrosis, eosinophils and lymphocytes without well-formed granulomas (10), **Figure 3**. It is evident that in GCM, correspondingly to CS, autoimmunity and dependence on CD4-positive T-lymphocytes are the backbones of pathogenesis (11). Similarly to CS, it is associated with Th-1/2 imbalance and impaired cytokine production (5,39). A gene expression study of GCM demonstrated up-regulation of all immune response genes studied, especially the ones involved in Th-1 activation (60). In murine models, experimental giant cell myocarditis can be produced by autoimmunization with myosin (61), and therapy with cyclosporine can prevent GCM in rats (62). However, autoantigens in humans remain poorly defined (63,64). Histological and immunohistochemistry findings in GCM have illustrated perivascular inflammatory infiltrates of giant cells and dendritic cells surrounding and consequently

obliterating small- and medium sized arteries, similarly to systemic forms of vasculitis (63). Moreover, in a study comparing plakoglobin (a cell-membrane protein involved in myocyte adhesion) expression in myocardial samples of patients with GCM, CS, arrhythmogenic right ventricular cardiomyopathy (ARVC), and lymphocytic myocarditis, expression was diminished in all except lymphocytic myocarditis, and all but lymphocytic myocarditis responded similarly to cytokine stimulation (65). This finding might serve as a potential link between these cardiomyopathies but warrants further studies (26).

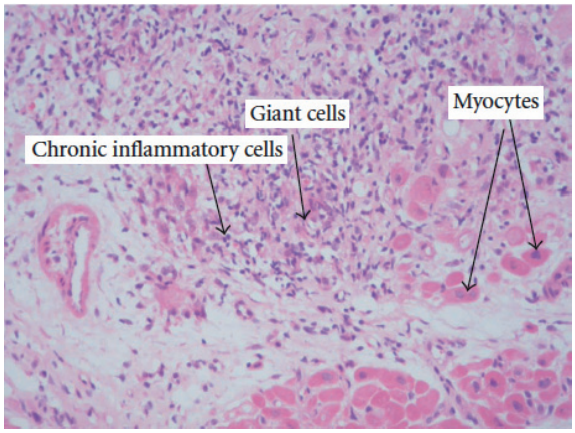


Figure 3. Light microscopic image of GCM. Adapted from (69).

Flu-like symptoms have been reported prior to the onset of GCM, making viral infection one possible trigger for the immunological cascade (7,27,66). Furthermore, drug hypersensitivity reactions have been attributed to the manifestation of GCM in rare cases, but the mechanism of this relationship is not clear (66-68). There are several similarities in the pathogenesis of GCM and CS, and it has been speculated that as a distinction between the two, the presence of eosinophils and their cytotoxic products might play an important role in GCM (11).

2.3 Epidemiology

2.3.1 Epidemiology of sarcoidosis

Valid sarcoidosis incidence and prevalence assessment is problematic and dependent on a variety of factors, including sampling bias and the absence of precise diagnostic methods (70,71). The reported numbers are based on lung sarcoidosis epidemiology and are highly variable. The overall clinically identified (i.e., symptomatic or incidental finding in chest radiography) incidence rate is estimated to be 5.0 per 100 000 person-years in the racially heterogeneous United Kingdom (72) and United States (73).

Reported annual incidence was higher in Scandinavia, 19 per 100 000 (74). A U.S. study comparing racial differences demonstrated an incidence rate of 10.9 per 100 000 in Caucasians and 35.5 per 100 000 in African Americans (75). In Finland, the prevalence of sarcoidosis is reported to be 28.2 per 100 000 and the incidence 11.4 per 100 000 (76). In an autopsy study among Japanese and American patients, the frequency of sarcoidosis was 39.3 per 100 000 autopsies in Japan and in 131.3 per 100 000 autopsies at Mayo Clinics in the U.S. (49). Another Japanese study estimated the incidence rate of sarcoidosis at autopsy to be 24 per 100 000 (16). The cumulative lifetime risk of sarcoidosis is 0.85% in white Americans (75) and 1.0% in men and 1.3% in women in Scandinavia (74).

Sarcoidosis commonly affects individuals in their prime, with incidence peaking at 20-40 years (31,75). Another incidence peak has been reported in some studies in females above 60 years of age (30,49,74). The female gender is generally associated with a slightly higher incidence (1.3-fold, 64 vs 36%) (31,75).

2.3.2 Epidemiology of CS

Myocardial sarcoidosis is commonly considered to affect 5% of patients with sarcoidosis (31,77-79). However, the reported prevalence varies greatly depending on the type of study: clinical, imaging, or autopsy report (80). Previous data on the prevalence of CS is scarce, but estimates can be derived from population-based studies of sarcoidosis (80). In a series of 181 patients with systemic sarcoidosis from Johns Hopkins Hospital, Baltimore, 11% had arrhythmias or conduction defects (31). In another U.S. study, symptomatic cardiac involvement was perceived in 2.3% (17 patients) of 736 patients with histologically confirmed sarcoidosis (30). Similarly, in a recent Swedish cohort, CS was diagnosed in 2.3% (23 patients) of 1017 biopsy-positive lung sarcoidosis patients, although CS was not histologically verified in all cases (81). CS accounts for 1.5-3% of all cardiac transplantations (82-84). The fact that up to 83-100% of patients undergoing cardiac transplantation due to CS were misdiagnosed for dilated cardiomyopathy (DCM) in pre-transplant investigations (84,85) reflects the difficulties in recognizing CS and thus complicates epidemiologic assessment. Scrutinized the other way around, among patients undergoing EMB due to unexplained cardiomyopathy, 2.5% (31 of 1235) were diagnosed with CS (86). Finally, since sudden cardiac death may be the first clinical manifestation in up to 25% of patients (87,88), determining the true incidence of CS would require comprehensive autopsy data. In a post-mortem study of 2950 consecutive routine autopsies, sarcoidosis was observed in 13 (0.4%) patients and myocardial sarcoidosis in two (0.006%) (89).

Nevertheless, accumulating data suggests asymptomatic sarcoid heart involvement to be much more common (1). Early autopsy studies revealed myocardial involvement in 20-27% of consecutive autopsied sarcoidosis patients, of whom 65% had previous history of heart failure and/or arrhythmias (15,17). Iwai et al. found cardiac

involvement post-mortem in as many as 67.8% (217 out of 320) of Japanese patients but only in 21.2% (14 out of 66) of black and in 13.6% (16 out of 117) of white patients (49). Therefore, the prevalence of CS in autopsy was 27 per 100 000 in Japan and 13 per 100 000 in the U.S. (49). Furthermore, sophisticated imaging studies have shown cardiac abnormalities in significantly higher proportions than suspected clinically. As presented in **Table 1**, silent cardiac involvement has been found by modern imaging modalities in 3.7-49% of patients with extracardiac sarcoidosis (77,90-96). The wide range of prevalence data is likely due to differences in imaging methods and patient selection (4). Based on both autopsy and imaging studies, the best estimate is that cardiac involvement is present in around 20-30% of patients with extracardiac sarcoidosis (71).

Table 1. Prevalence of imaging findings compatible with CS in patients with sarcoidosis screened for cardiac involvement.

Study	N, patients	Patients with cardiac involvement*	Imaging modality	Other important findings
Nagai et al. 2014	61 Patients were JMWH 2006 criteria negative	13%	LGE-CMR	LGE did not predict adverse events in patients with no cardiac symptoms and normal EF
Greulich et al. 2013	153	26%	LGE-CMR	CMR findings were best predictors of cardiac adverse events
Patel et al. 2011	152	19%	LGE-CMR	Involved only patients with preserved EF (≥ 50%)
Patel et al. 2009	81	26%	LGE-CMR	CMR twice as sensitive as JMWH criteria
Mehta et al. 2008	62	39%	PET and/or LGE-CMR	21% had cardiac symptoms
Smedema et al. 2005	101	3.7%¶ 84%§	CMR, thallium scintigraphy	Cardiac involvement may well be present without ECG abnormalities
Dhôte et al. 2003	50	35% 100% (7/7)	CMR	All patients with cardiac symptoms had inflammatory exudative CMR finding ° compared to 35% in patients without symptoms

Vignaux et al. 2002	40	49% 100%	CMR and thallium scintigraphy	CMR abnormalities were very similar in symptomatic and asymptomatic patients

*Defined as imaging findings compatible with CS. ¶ Without cardiac symptoms. § With cardiac symptoms. ° Defined as T1 and T2 hypersignal.

LGE= late gadolinium enhancement, CMR= cardiac magnetic resonance imaging, JMHW= The Japanese Ministry of Health and Welfare, PET= positron emission tomography, ECG= electrocardiogram.

As with systemic sarcoidosis, many CS studies have demonstrated a female predominance (5,16,97), yet there are several studies indicating a slight male predominance (22,77,98,99). The female majority was the only characteristic differentiating CS from other causes of heart failure in 825 patients undergoing cardiac transplantation (50% females in CS vs 22.8% in non-sarcoidosis group) (82). Similarly, in patients with a clinical picture of DCM, female dominance was the distinguishing feature between CS and idiopathic DCM (100). The average age at diagnosis in CS is similar to systemic disease, being 36-53 years (77,97,101).

2.3.3 Epidemiology of GCM

GCM is rarer than CS, but representative epidemiologic lifetime data is lacking. In two autopsy registries from Japan and England, 25 out of 377 841 (6.6/100 000) and three out of 12 815 (23.4/100 000) cases respectively, were classified as GCM (25,102). In a Finnish autopsy registry study, 5.6% (8/142) of myocardial autopsy samples initially classified as myocarditis in death certificates were identified as GCM in histopathological reanalysis (103). Among 174 consecutive patients with symptoms of clinical myocarditis, GCM was found in 3% (5 patients) (64). In two series of 424 and 4738 patients with idiopathic DCM biopsied for suspected myocarditis, GCM was documented in 0.2-1.2% (5 and 10 patients) and CS in 0.9-1.2% (4 and 10 patients), respectively (9) (104). In a series of 340 patients undergoing cardiac transplantation, GCM was observed in seven explanted hearts (2%) (37).

The average age in GCM does not differ from CS, being around 42 years and ranging from 16 to 69 years 1(7,11,63). In rare cases GCM can occur in children (105,106) and younger patients are reported to have a worse outcome (64,107). In the largest GCM report, both genders were equally affected by GCM (5), whereas other smaller studies have demonstrated minor female or male predominance (27,63). The spatial and familial clustering observed in CS has not been demonstrated in GCM (5).

2.4 Clinical manifestations

Lung sarcoidosis is a disease with a tendency towards natural healing and spontaneous remission in nearly two-thirds of patients (79). In contrast, manifest involvement of the heart is notorious for poor outcomes. CS may precede, follow or occur concurrently with involvement in the lungs or other organs and the degree of extracardiac involvement does not correlate with cardiac involvement (93,108). The clinical manifestations are related to the location and extent of granulomatous involvement in the heart (13). Myocardium and epicardium are the most commonly affected parts of the heart, but granulomas can also involve the pericardium, endocardium, and rarely the valves (16,87). Typical locations are the basal parts of the left ventricle (LV), the interventricular septum and ventricular free wall, followed by the right ventricle (RV) and the atria (16,87,109), as presented in **Figure 4**.

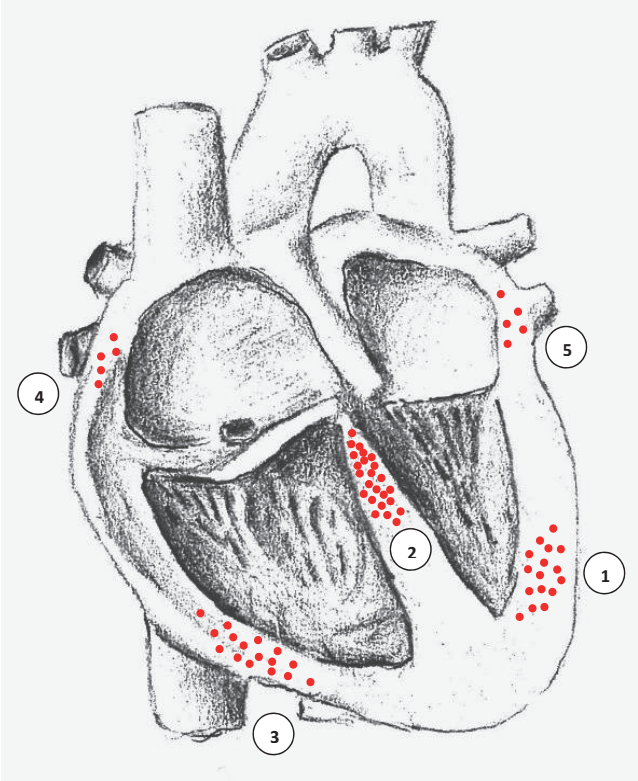


Figure 4. The most common sites of sarcoid granulomas in the heart**.** Modified from (35,109).

1) Left ventricular free wall (96%)
2) Interventricular septum (73%)
3) Right ventricular free wall (46%)
4) Right atrium (11%)
5) Left atrium (7%)

Patients with CS may present with a wide range of symptoms and signs, ranging from asymptomatic electrocardiographic or imaging findings to sudden death (88,110). The typical symptoms are nonspecific and include palpitations, presyncope or syncope, and symptoms of ventricular dysfunction. A summary of the different manifestations reported in the literature is presented in **Table 2**. The three principal sequelae of CS are 1) conduction abnormalities, 2) ventricular arrhythmias, and 3) heart failure (4).

Table 2. Clinical manifestations of CS and their prevalence.

Study				Manifestations of CS						
Authors	n	Type	CS criteria	3° AV-block	RBBB	LBBB	Arrhythmias Atrial	Arrhythmias Ventricular	SCD	CHF
Forbes et al. 1962	25	Autopsy	Autopsy confirmation						20%	
Matsui et al. 1976	42	Autopsy	Autopsy confirmation	35%	43%	8%		13%		10%
Roberts et al. 1977	113	Autopsy	Autopsy confirmation						11%	
Silverman et al. 1978	23	Autopsy	Autopsy confirmation						35%	
Fleming et al. 1986	250	Clinical and autopsy	Vast majority with histologic evidence	20%			9%	19%	15%	
Yazaki et al. 1998	15	Clinical	11 with histological and 4 with clinical confirmation		57%	0%			12%	26%
Yazaki et al. 2001	95	Clinical	JMHW 1993	45%				18%		48%
Okura et al. 2003	42	Clinical	Histologically verified	26%				31%*		
Chapelon-Abric et al. 2004	41	Clinical	Own criteria, all with extracardiac confirmation	7%			19%	11%		
Smedema et al. 2005	19	Clinical	JMHW 1993	16%						57%
Mehta et al. 2008	24	Clinical	Clinically diagnosed		8%	4%		17%		13%
Schuller et al. 2011	52	Clinical	Modified JMHW		23%	4%				

Schuller et al. 2012	112	Clinical	Modified JMHW	15%	27%			32%¶		
Cain et al. 2014	44	Clinical	LGE-CMR positive, biopsy-proven extracardiac sarcoidosis				36%			
Willner et al. 2014	100	Clinical	With histologic Confirmation				32%			
Kron et al. 2015	235	Clinical	Also clinically diagnosed included		27%	5%		36%¶		
Nagai et al. 2015	83	Clinical	Modified JMHW	40%						13%

*26% VT, 5% VF. ¶Appropriate ICD therapy for VT/VF.

CS= cardiac sarcoidosis, 3° AV-block= third degree AV-block, LBBB= left bundle branch block, RBBB= right bundle branch block, SCD= sudden cardiac death, CHF= chronic heart failure, JMHW= Japanese Ministry of Health and Welfare, VT= ventricular tacycardia, LGE-CMR= late gadolinium enhancement cardiac magnetic resonance imaging.
Numbers presented either at presentation or during disease course.

2.4.1 Conduction abnormalities

Conduction abnormalities are the most common manifestations of CS due to the affinity of granulomas for the atrioventricular septum and the vulnerability of the conduction system to granulomatous infiltration (111). Yet another often mentioned, yet probably rare mechanism for conduction disturbances is granulomatous infiltration of the nodal artery causing ischemia of the conduction system (112).

Virtually any kind of conduction abnormality is possible in CS from bundle branch blocks to AV-blocks of any degree (110). Different conduction abnormalities are reported in 42-65% of patients (5,16,22,77,93,97-99,101,113,114). Complete AV-block, present in 15 to 45% in the larger studies, is the most frequent symptomatic first manifestation in CS (5,16,97,115). AV-block due to sarcoidosis occurs at a younger age compared to patients with AV-block of other causes (116). In a Japanese study, clinically or histologically verified CS was diagnosed in 11.2% of patients manifesting with high-degree AV-block (117). Bundle branch blocks are present in 27-57%, with right bundle branch block (RBBB) being the more frequent finding (113,114).

2.4.2 Arrhythmias

Active inflammation may lead to increased automaticity and fibrous granulomatous scars, promoting re-entry substrates by creating slow conduction zones in myocardium. Furthermore, the variable involvement of LV and/or RV is a characteristic feature of CS. The combination of these elements makes ventricular arrhythmias in CS not only common, but also challenging to treat (98,118-120). Ventricular arrhythmias in CS range from asymptomatic ventricular premature beats (VPBs) to ventricular fibrillation (VF). As in other types of structurally abnormal hearts, the VTs usually result from scar-induced re-entry mechanisms, with the most common mechanism likely to be macro-reentrant arrhythmias around granulomatous scars (121,122). The distinguishing feature in CS is a higher prevalence of electrical storm, in which active inflammation can play a significant role (123). An electrical storm (defined as ≥ 3 appropriate ICD therapies in 24 hours) occurred in 14% (16/112) of CS patients in a 2.4-year follow-up after an ICD implantation (98). Characteristically, CS-associated VT is monomorphic with either LBBB or RBBB morphology, but polymorphic VTs are also possible (98,119,124,125). Electrophysiological studies have demonstrated that most VTs originate from confluent regions of RV and LV scarring with predilection to the septal and peritricuspid areas (122,126). One additional possible etiology for VTs in CS is Purkinje fiber-related VT, due to conduction disturbances (118). In the study by McArdle et al. (127), sustained VTs were associated with signs of active inflammation by PET, while Banba et al. (124) did not find a link between disease activity and arrhythmias. In patients dying suddenly from CS, histologic lesions more often contained well-formed granulomas instead of early inflammatory cell lesions or healed collagen-rich lesions (87).

Variable ventricular tachyarrhythmia incidence numbers of 11% to 31% have been previously reported based on autopsy and clinical follow-up studies in CS (5,16,22,93,97,101). Recently, pacemaker follow-up studies have yielded more precise incidence numbers. In a rather selected CS population from an academic center with ICDs implanted either for secondary prevention (with history of VT, VF, sudden cardiac arrest, or syncope of unknown etiology, n=16) or primary prevention (without aforementioned history, n=29), the incidence of appropriate ICD therapies (shock and/or anti-tachycardia pacing) was 15% per year and there was no significant difference in the incidence rate between the two groups (128). Two larger studies presented corresponding ICD therapy rates of 36.2% (85/234) and 32.1% (36/112) in mean follow-ups of 4.2 years (129) and 2.4 years (98). In these two series, secondary prevention patients had higher rates compared to primary prevention patients. Overall, the frequency of appropriate ICD therapies and hence ventricular arrhythmias was calculated to be annually 9-15% (4), being much higher than in landmark studies of heart failure (88)3. The true incidence of sudden cardiac death in CS is not known, but currently up to 25% of patients with CS are estimated to present with sudden death (16,17,88,113,130). From an electrophysiologist's point of view, CS revealed to be the etiology in 5-28% of patients referred to electrophysiological study and/or catheter ablation due to monomorphic VT of unknown cause (121,126,131). CS derived VTs were inducible in electrophysiological studies in virtually all patients (119,121,132).

Given their diverse pathophysiology, ventricular arrhythmias may manifest unexpectedly, though certain predisposing factors have been identified. Logically, LV dysfunction was the strongest predictor of arrhythmias in all studies, but still a significant number of patients with appropriate therapies had LV ejection fraction (LVEF) of >35% (98,128,129). Besides reduced LVEF, male gender (129), history of syncope (129), longer follow-up (128), higher prevalence of complete heart block (128), and RV dysfunction (RVEF≤45%) predicted future VT events (98).

A few studies have addressed the usefulness of programmed ventricular stimulation in identifying CS patients with a risk of VTs. Mehta et al. performed programmed ventricular stimulation in 76 patients with biopsy-proven systemic sarcoidosis, with evidence of CS in PET/CMR but without cardiac symptoms (133). Eight out of 76 (11%) were inducible for VTs and received an ICD. Over a median follow-up period of five years, two out of eight died and four out of eight had appropriate ICD shocks, compared to no arrhythmic events in the non-inducible patients. Likewise, in a study of Aizer et al., 32 CS patients underwent programmed ventricular stimulation and patients with either spontaneous (n=6), or inducible (n=6) VT received an ICD (134). In a follow-up, after 2.7 years, five out if six patients with spontaneous VTs and four out of six patients without spontaneous but with inducible VTs received appropriate ICD therapies. Thus, stimulation identified patients with future arrhythmic events and no arrhythmic deaths occurred in these patients with either spontaneous or inducible VT when all of them received an ICD. However, the electrophysiological study was not fully sensitive, as two

(10%) patients without spontaneous or inducible sustained ventricular arrhythmia suffered sudden death or sustained VT on follow-up. Interestingly, in another smaller study of 16 patients, the inducibility of VT/VF did not predict future ICD therapies (134).

Due to overlapping clinical presentation, patients first presenting with ventricular arrhythmias may be misdiagnosed as ARVC and may even fulfill the Task Force ARVC criteria (121,135-137). The distinguishing features in this respect are lack of conduction abnormalities and positive family history in ARVC, compared with more frequent septal involvement and LV dysfunction in CS (138). The disruption of desmosomal proteins may be a potential link between these two arrhythmogenic diseases (65).

Supraventricular arrhythmias occur in 9-36% of CS patients (4,22,101,139-141). The causes of atrial arrhythmias are diverse, with direct atrial granulomatous infiltration causing abnormal automaticity, triggered activity and re-entrant circuits as speculated mechanisms (4,123,140). Another possible cause for atrial arrhythmias is elevated atrial pressure due to ventricular dysfunction or pulmonary hypertension (140,142). Atrial fibrillation is the most common supraventricular arrhythmia in CS, followed by atrial tachycardia and atrial flutter (123,140). Left atrial enlargement was the only variable found to be associated with supraventricular arrhythmias in CS (risk ratio, 6.12; 95% CI, 2.19-17.11) (137).

2.4.3 Heart failure

Granulomatous infiltration of the myocardium with the resulting inflammation and fibrosis can lead to either systolic or diastolic dysfunction or a combination of the two. Diffuse cardiac involvement leads to global dysfunction, whereas focal involvement produces segmental hypokinesia and aneurysms independent of coronary circulation zones (143,144). Congestive heart failure with reduced LV systolic function is a sign of a widely advanced disease state (71). Besides extensive myocardial infiltration, pump failure may result from right ventricular overload caused by pulmonary involvement or from atrioventricular valve regurgitation caused by papillary muscle involvement (145).

Prior to current diagnostic methods, overt heart failure was a common first manifestation of CS with reported prevalence varying from 25 to 75% (22,71,97,116). Today, as diagnosis is made at earlier stages, around 13-18% of patients have systolic dysfunction at diagnosis (93,146). CS is frequently misdiagnosed as DCM. In two Japanese studies with patients undergoing left ventriculoplasty for suspected DCM, CS was found in 3.6-7% (14/384 and 8/110, respectively) (100,147). In a third Japanese study involving 533 patients with non-ischemic cardiomyopathy undergoing cardiac resynchronization therapy (CRT), 4.5% (25 patients) had CS (148). The evolution of LVEF during disease course is highly variable and data on its natural course is lacking.

2.4.4 Other manifestations

Direct valve involvement in CS is relatively uncommon, but case reports involving all four valves have been reported (110,149,150). In the study by Fleming et al. valve lesions were reported in 8.4% of patients (22). The most common valve dysfunction in CS is mitral regurgitation secondary to either papillary muscle infiltration or LV dilatation (143,151). Symptomatic pericardial involvement is very rare, but mild pericardial effusion has been reported in 19% patients (22,152-154). Constrictive pericarditis as a manifestation of CS has also been reported (155).

Clinical involvement of the coronary arteries is possible but rare in CS. Despite this, granulomas have been detected within atherosclerotic plaques and within the media and peri-arterial tissue (156). Thallium perfusion scans have shown ischemic findings in the absence of stenoses in coronary angiographies (157,158). Microvascular spasm has been speculated as a possible explanation for the ischemic findings (156,159). Another mechanism could be sarcoid coronary vasculitis (160,161). ST-segment elevation is a rare finding in CS and is considered to be due to the presence of myocardial inflammation/fibrosis and abnormal wall motion, including ventricular aneurysm. In some cases ST-elevations may be transient and may attenuate after steroid treatment (162). With sarcoidosis being a systemic inflammatory disease, up to over half of all CS patients are reported to experience constitutional symptoms like fatigue (101,163). Finally, in its early stages with minor myocardial involvement, CS can be a clinically fully silent disease.

2.4.5 Isolated cardiac sarcoidosis

Sarcoidosis can manifest with solely cardiac symptoms. Previously, there was a misunderstanding that the heart is mostly affected in widespread systemic sarcoidosis. Yet, already in the early autopsy study from Roberts et al., the authors concluded that most patients with CS have little or no clinical evidence of dysfunction of extracardiac organ systems (109). Furthermore, it was demonstrated that the extent of extracardiac sarcoidosis did not differ among patients with mild, severe or no cardiac disease (17). In a more recent autopsy study from the U.S., 40% of 25 CS patients did not have gross extracardiac involvement (87). On the contrary, in a Japanese autopsy series of 42 CS patients, extracardiac sarcoidosis involvement was found in literally all patients, involving lymph nodes in 100%, lungs in 88%, liver in 64% and spleen in 43% (16). Thus, even in autopsy studies the reported frequency of extracardiac involvement is highly variable and clinical studies are even more prone to differences, depending on the precision of the examination. In a Finnish cohort of nine CS patients, eight had clinically isolated CS and one developed lung manifestation later (119). Recently, Blankstein et al. reported extracardiac involvement defined as abnormal FDG uptake in whole-body 18F-FDG-PET in 26% of 71 CS patients (108). In contrast, Tezuka et al. found extracadiac involvement after work-up including chest-computed tomography,

whole-body 18F-FDG-PET, ophthalmologic and dermatologic examinations in 73% (30/41) of CS patients (164). Similarly, in the study from Kron et al., 94.5% of 235 CS patients had clinically and/or histologically detected extracardiac sarcoidosis (99). Although the differences in the proportion of patients with extracardiac involvement might partially result from differences in genetic and environmental factors, the varying extent of extracardiac work-up is the most probable explanation for the divergence (16,49,99,164).

There are three possible clinical scenarios of isolated CS: 1) sarcoid lesions appear first in the heart and involve other organs later in the course of the disease, 2) a truly isolated cardiac involvement exists, and 3) sarcoid lesions are present in other organs but are undetectable by standard examination methods (165). The critical issue in isolated CS is the difficulty of its diagnosis, given the limited sensitivity of EMB (2,21,165). As one example, isolated CS may present clinically as DCM and distinguishing them can be problematic. In a study of 15 patients with CS and 30 with DCM, both with LVEF around 30%, the disjunctive features were female predominance (73% vs 20%), high incidence of AV-block (67% vs 0%), RBBB (57% vs 17%), abnormal wall thickness (73% vs 17%), and uneven wall motion abnormalities in LV angiography (113). As an additional detail, sometimes the disease course of CS may mimic postpartum cardiomyopathy, which has to be recalled in differential diagnostics in pregnancy and postpartum reckoning that CS commonly affects young females (31).

2.4.6 GCM

GCM is known as a myocardial disease characterized by acute onset, progressive heart failure, and has a frequently fatal outcome if untreated. In the two largest, multicenter studies of 63 and 73 GCM patients from overlapping groups, 64-75% of patients presented with congestive heart failure (5,11). Other first manifestations in these landmark studies were ventricular arrhythmias in 14-32%, signs and symptoms mimicking acute myocardial infarction in 6-19% and complete heart block in 5-15%. In a later study of the same group, presentation with heart failure was less common (in 45%, five out of 11) and mean LVEF at baseline only moderately reduced (44±18%) (107). In two small cohorts of five and eight patients respectively, mean LVEF was markedly reduced, 37% at presentation (9,63). In a study addressing long-term outcome, during the mean follow-up of 5.5 years starting one year after GCM diagnosis, 50% (13 out of 26) patients had new or worsening episodes of heart failure, suggesting disease recurrence (166). During the course of the disease, 23-60% of the patients develop ventricular arrhythmias that are often pleiotropic and can be refractory to treatment (5,9,11,107,167). Follow-up ventricular arrhythmias occur primarily in patients who initially presented with ventricular arrhythmias (166). AV-block has been reported in combination with heart failure and VTs (5,9,11), but also as the sole manifestation of GCM (8). As an additional detail, a distinct clinicopathologic entity involving only the atria and entailing a more favorable prognosis compared to classic

ventricular GCM has been described (168). Finally, GCM can present in two different forms; either as an isolated myocardial disease or more rarely together with skeletal muscle involvement, called thymoma-associated giant cell polymyositis (66,169-171).

The presentation of GCM is highly variable. Its progression is often rapid, with time from symptoms onset to hospital admission ranging from two to six weeks in the larger series (5,11,63). Even so, the manifestation of GCM can vary widely from sudden death (27), to a more indolent course with mild DCM progressing to end-stage in nine years (9,37,172). Ren et al. described three cases with a protracted disease course with time from active myocarditis episode to transplantation or death being 5-10 years without immunosuppression (8). Recently, Maleszewski et al. reported prolonged survival of over 19 years beyond initial diagnosis with immunosuppression (166). In a study comparing GCM with CS, presentation with heart failure predicted GCM and presentation with AV-block or more than nine weeks of symptoms were in favor of CS (5). Taken together, CS and GCM may present with similar symptoms and differentiating them on the basis of clinical symptoms is not possible (5,9). The distinctions in histology and outcome are discussed elsewhere in this thesis.

2.5 Diagnostics of CS and GCM

An unequivocal diagnosis of CS is based on a clinical presentation compatible with myocardial disease associated with histology of non-caseating granulomatous inflammation in heart muscle. A probable but clinically sufficient diagnosis comes from extracardiac histology of sarcoidosis combined with clinical manifestations and cardiac imaging indicative of myocardial involvement. In cases of clinically isolated cardiac involvement, the diagnosis can be extremely difficult and rest on a high index of suspicion. Still today, in 57-100% of patients undergoing transplantation due to end-stage heart failure, the diagnosis is missed until examination of the explanted heart (82,84,173). The current guidelines of the American Thoracic Society/European Respiratory Society recommend screening for asymptomatic CS in patients with sarcoidosis diagnosis from other organs (174). Several groups have published stepwise diagnostic algorithms to aid clinicians in CS diagnostics (4,28,165,175-177). **Figure 5** presents a simple recently published screening strategy (4).

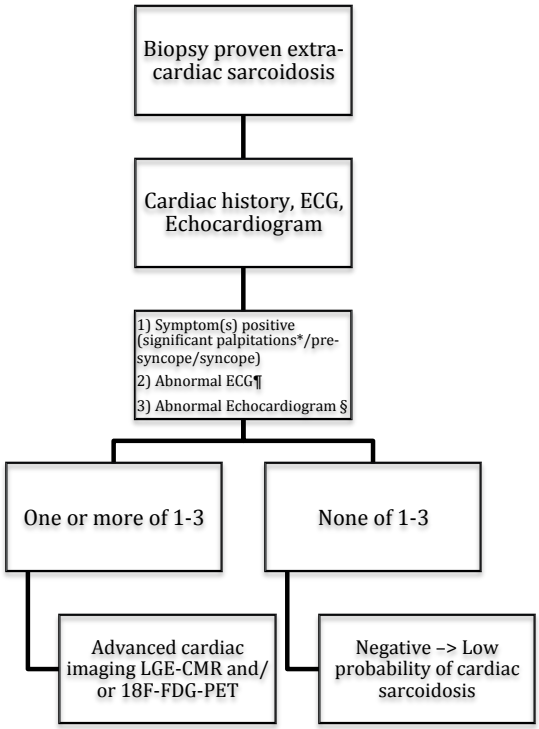


Figure 5. Suggested study algorithm of patients with biopsy-verified extracardiac sarcoidosis. Adapted from (4).

*Palpitations were defined as "prominent patient complaint lasting >2 weeks".
¶Abnormal electrocardiogram defined as complete left or right bundle branch block and/or presence of unexplained pathological Q waves in two or more leads and/or sustained 2nd or 3rd degree AV-block and/or sustained or non-sustained VT.
§ Abnormal echocardiogram defined as LVEF <40% and/or basal septum thinning and/or regional wall motion abnormality.

2.5.1 Diagnostic guidelines and recommendations in CS

In Japan, CS has long been a target of research and *the Japanese Ministry of Health and Welfare* (JMHW) published guidelines for diagnosing CS in 1993 (175), **Table 3.** Since then, the JMHW criteria have been widely applied as the standard diagnostic tool against which other diagnostic modalities have been compared. However, these guidelines are not properly validated for either clinical or research applications (13). In 2006 the guidelines were revised; histological confirmation was no longer required in "clinical diagnosis" group and LGE-CMR was included as a minor criterion (178). It is of note that 18F-FDG-PET was still not included. It has become evident that the JMHW guidelines have too low a sensitivity in detecting CS, especially its early manifestations

(1,2). Merely the fact that JMHW guidelines emphasize abnormal electrocardiogram (ECG) results, yet even in patients with extensive myocardial lesions in autopsy, the pre-mortem ECG may be normal in 25%, makes these guidelines too insensitive (17,91). Recent advances in imaging methods highlight this inaccuracy. As an example, in the study by Patel et al., the JMHW criteria found only 52% of sarcoidosis patients with LGE in CMR (92). In some recent studies, patients with pulmonary sarcoidosis and cardiac abnormalities in LGE-CMR have been defined as having CS even without clear CS symptoms (chest pain, palpitations or no cardiac symptoms at all), which is also a somewhat problematic definition (179).

Table 3. Guidelines for diagnosing CS from the Japanese Ministry of Health and Welfare 1993 (175).

1. Histologic diagnosis group

CS is confirmed when histologic analysis of operative or EMB specimens demonstrates epithelioid granuloma without caseating granuloma.

2. Clinical diagnosis group

In patients **with a histologic diagnosis of extracardiac sarcoidosis**, CS is suspected when item (a) and one or more of items (b) through (e) are present:

a) Complete RBBB, left axis deviation, AV-block, VT, VPBs (≥ Lown 2*), or abnormal Q or ST-T segment change on the ECG or ambulatory ECG
b) Abnormal wall motion, regional wall thinning or dilatation of the LV
c) Perfusion defect by Thallium-201 myocardial scintigraphy or abnormal accumulation by gallium-67 or technetium-99m myocardial scintigraphy
d) Abnormal intracardiac pressure, low cardiac output, or abnormal wall motion or depressed LVEF
e) Interstitial fibrosis or cellular infiltration over moderate grade, even if the findings are nonspecific

CS= cardiac sarcoidosis, EMB = endomyocardial biopsy, RBBB= right bundle branch block, AV-block= atrioventricular block, VT= ventricular tachycardia, VPB= ventricular premature beat, *30 or more VPBs per hour, ECG= electrocardiogram, LV= left ventricle, EF =ejection fraction.

In 2014, an expert group of the Heart Rhythm Society published consensus recommendations for diagnostic criteria, diagnostic pathways in different clinical scenarios and risk stratification in CS **Table 4** (4). In the same year, the World Association of Sarcoidosis and Other Granulomatous Disorders updated an instrument based on expert consensus for assessing different organ involvement in sarcoidosis (177). The tool classified organ involvement into three categories; highly probable (>90% likelihood), probable (50-90% likelihood), and possible (<50% likelihood). In clinical studies, considerably variable inclusion criteria for CS have been applied.

Table 4. Expert consensus recommendations on criteria for the diagnosis of CS. Adapted from (4).

1. Histological diagnosis from myocardial tissue
   CS is diagnosed in the presence of non-caseating granuloma on histological examination of myocardial tissue with no alternative cause identified (including negative organismal stains, if applicable).

2. Clinical diagnosis from invasive and non-invasive studies:
   It is probable* that there is CS if:
   a) There is a *histological diagnosis of extracardiac sarcoidosis*
      and
   b) One or more of following is present
      - Steroid +/- immunosuppressant responsive cardiomyopathy or AV-block
      - Unexplained reduced LVEF (<40%)
      - Unexplained sustained (spontaneous or induced) VT
      - Mobitz type II 2nd or 3rd degree AV-block
      - Patchy uptake on dedicated cardiac PET (in a pattern consistent with CS)
      - Late gadolinium enhancement on CMR (in a pattern consistent with CS)
      - Positive gallium uptake (in a pattern consistent with CS)
      and
   c) Other causes for the cardiac manifestation(s) have been reasonably excluded

* In general, "probable involvement" is considered adequate to establish a clinical diagnosis of CS.

2.5.2 Diagnostic examinations and procedures

2.5.2.1 ECG and Holter monitoring

According to Mehta et al., the 12-lead ECG has a high specificity of up to 97%, but low sensitivity in detecting CS in patients with systemic sarcoidosis (93). In a cohort of 112 biopsy-proven pulmonary sarcoidosis patients with symptoms suggestive of CS, RBBB was found in 23% of patients with cardiac involvement by modified JMHW criteria vs only in 7% of non-CS patients (114). Likewise, there was a much higher prevalence of fragmented QRS complex with cardiac involvement (75% vs 34%) (146). Other ECG findings associated with CS are any degree of AV-block, frequent atrial or ventricular ectopic beats, and pathologic Q-wave or ST-T changes indicating local myocardial infiltration (123). More delicate ECG tools, such as microvolt T wave alternans and signal-averaged ECG, are more sensitive, with 56-75% and 79-96% positive and negative predictive values in CS diagnosed by JMHW`s early or revised criteria (146,180).

In studies focusing on screening sarcoidosis patients for cardiac involvement, Holter monitoring has shown abnormalities in 50-67% of CS patients (diagnosed by early- or revised-, or modified to include LGE-CMR, JMHW criteria) with specificities ranging from 21 to 97% (93,125,181). Nevertheless, the abnormalities observed are nonspecific including VPBs and non-sustained VTs. Suzuki et al. detected ≥ 100 VPBs in 67% (8/12) of CS patients, 8% (2/26) of sarcoidosis patients, and in 5% (3/58) of healthy controls (181). The sensitivities and specificities of individual tests in three studies are presented in **Table 5**.

Table 5. Sensitivities and specificities of diagnostic studies and findings in CS. Adapted from (93,125,146).

Abnormality at baseline	Sensitivity (%)	Specificity (%)
ECG	8 - 26	64 - 97
Signal-averaged ECG (any abnormal domain)	28 – 52	80 - 82
Holter	50 – 89	21 – 97
EP study	40	71

CS= cardiac sarcoidosis, ECG = electrocardiogram, EP =electrophysiologic

2.5.2.2 Echocardiography

Given its wide availability and noninvasiveness, two-dimensional transthoracic echocardiography is the primary imaging screening tool for CS, but the results can be normal in over two-thirds of patients at diagnosis (93,182). More specifically, echocardiography commonly fails to detect early, mild or localized disease (1). Echocardiogaphic abnormalities are nonspecific, with LV systolic and diastolic dysfunction, wall motion abnormalities in a non-coronary distribution, and septal abnormalities as the most common findings (93,113,183). Septal abnormalities, both thickening due to infiltration or edema and thinning due to scarring, are characteristic of CS and constitute one of the strongest predictors of cardiac involvement in patients with systemic sarcoidosis (90) (**Figure 6**).

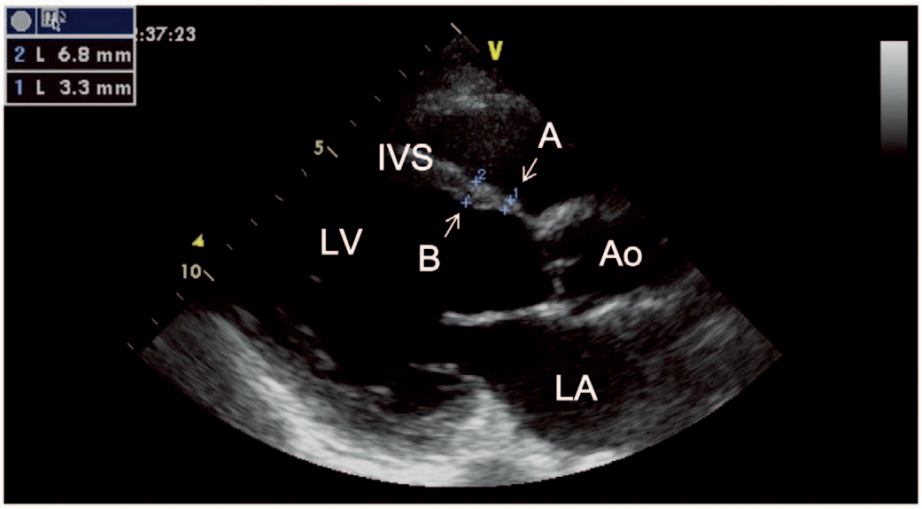


Figure 6. Echocardiographic image of CS with basal thinning. IVS=interventricular septum, A= point located 10mm from the aortic annulus, i.e. basal IVS, B= IVS at the standard level of the mitral valve leaflet tips, Ao=aorta, LA=left atrium, LV=left ventricle. Adapted from (184).

Several studies examining patients with pulmonary sarcoidosis have demonstrated that diastolic dysfunction and subnormal systolic function (evidenced by tissue doppler measurements, isovolumic acceleration etc.) are more common compared to healthy controls (163,182,185-188). These findings can be related to subclinical myocardial involvement but the abnormalities are subtle and cannot usually be detected by traditional echocardiographic methods (189). As the conventional two-dimensional echocardiography lacks sensitivity to evaluate CS, new more sensitive methods are needed (190). Recently, speckle-tracking imaging which assesses the intrinsic deformation (strain) of the myocardium, has shown promise in this respect (80). In a study of 39 patients, 3-D speckle-tracking radial strain showed good potential to distinguish CS (diagnosed by revised JMHW criteria) from DCM (191). Furthermore, in a study of 96 biopsy-proven systemic sarcoidosis patients without abnormalities in conventional echocardiography, the LV global longitudinal strains were lower compared to healthy controls (192). Finally, in a study of 100 systemic sarcoidosis patients and 100 healthy controls, the average global longnitunidal strain was lower in sarcoidosis and also a prognostic marker for future adverse cardiac events (80).

Echocardiography has important prognostic value, as LV dysfunction and dilatation are strong prognostic markers for future adverse cardiac events (80,97). Finally, echocardiography is the primary follow-up tool for assessing the development of LV dysfunction.

2.5.2.3 LGE-CMR

LGE-CMR has become the preferred imaging modality in the diagnosis of CS (1,77,90). CMR offers both structural and functional information without radiation exposure (1). Even minor areas of myocardial damage can cause LGE at CMR, which thus enables early diagnosis prior to LV deterioration (1). The structural abnormalities encountered in CS include local wall motion and thickness alterations that predominantly involve the septum and may sometimes result in aneurysm formation (164,165) (**Figure 7**). CMR also aids the precise assessment of LV function and chamber sizes. In CS diagnostics, the sensitivity and specificity of LGE-CMR compared to moderated JMHW guidelines were 75-100% and 77-92%, with positive and negative predictive values of 55% and 100%, respectively (164,193-195). In a cohort of 115 CS patients (with cardiac biopsy confirmation or extracardiac biopsy confirmation and electrophysiological disturbances compatible with CS), LGE-CMR findings were normal in 14% and the septum was commonly affected (129).

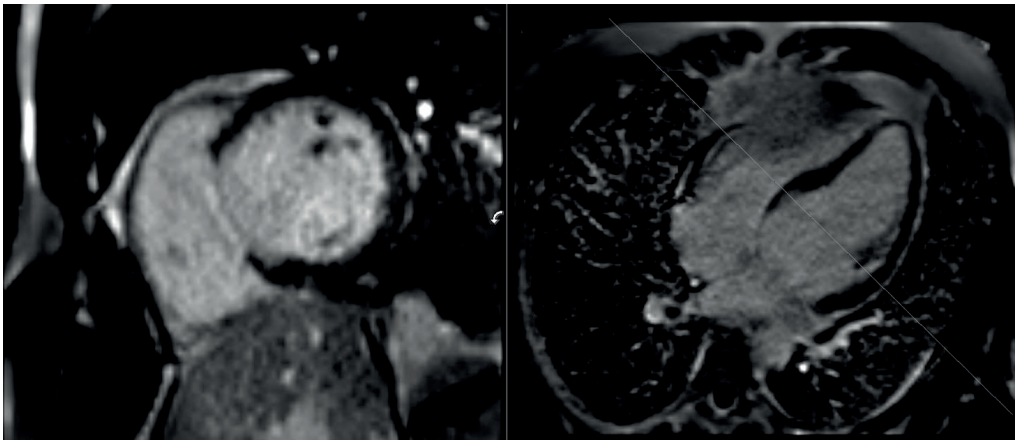


Figure 7. Short axis and four chamber CMR image of CS with basal septal thinning and myocardial late gadolinium enhancement. (With permission from Dr. Miia Holmström, HUS Medical Imaging Center, Radiology, University of Helsinki and Helsinki University Hospital).

The physiological mechanism of myocardial LGE is as follows. The contrast agent, gadolinium chelate, is a biologically inert tracer that distributes freely to extracellular space but does not cross intact cell membranes (196). In damaged myocardium, the combination of increased extracellular volume due to edema and slower washout kinetics results in an accumulation of the tracer seen as gadolinium enhancement (196). LGE imaged 10-15 minutes after contrast injection is the cornerstone finding in myocarditis such as CS, visualizing myocyte necrosis and fibrosis (197). The most typical LGE pattern in CS consists of multiple, patchy mid-myocardial lesions in a non-

coronary (i.e., sparing the endocardium and localizing independently of any coronary circulation area) distribution with septal and RV involvement (91,96). Yet, no specific pattern of LGE is pathognomonic for CS (4,195). Even subendocardial enhancement in a coronary distribution resembling ischaemic heart disease has rarely been observed (90). It is recommended that the CMR studies be interpreted by a specialized radiologist and integrated with the clinical data (4).

While LGE imaging can help in distinguishing patterns of myocyte necrosis and fibrosis from ischemic injury, T2-weighted and early gadolinium enhancement imaging (measured rapidly after contrast) indicate other inflammatory processes such as edema, capillary leakage and hyperemia (198). Active sarcoidosis lesions in the heart muscle are characterized by focal wall thickening due to infiltration and edema, combined with wall motion abnormalities, increased signal intensity on T2-weighted images, and early gadolinium enhancement (94,193). In the chronic phase, wall thinning and delayed gadolinium enhancement are seen as signs of scarring and fibrosis (199,200). In reality, these two phases commonly overlap (145). Despite its high sensitivity, CMR may miss early inflammation and differentiating active inflammation from fibrosis can be problematic (1,198).

In addition to its diagnostic utility, contrast CMR is a potent risk stratification tool, with increasing evidence from several studies showing that the presence and magnitude of LGE predict future adverse cardiac events in patients with systemic sarcoidosis (91,95,96,201) and in patients with CS (202-204). Despite this, one recent study involving mostly lung sarcoidosis patients, all without cardiac symptoms and preserved LVEF, suggested the contrary as there was no difference in cardiac events in LGE positive and negative groups (90). Transmural LGE lesions and a higher number of affected segments are associated with larger ventricles and lower LV ejection fractions (96,196,205). Furthermore, LGE-CMR has been shown to be useful in the assessment of steroid response and targeting EMB (1,95,203,206).

As CMR studies are booming and new, even more sensitive methods such as multicontrast late-enhancement are developed (207), more sarcoidosis patients without cardiac symptoms but with positive CMR findings are likely to be found. There is no consensus on the treatment strategy of these patients (197).

2.5.2.4 18F-FDG-PET

18F-FDG-PET is another advanced imaging method used in the diagnosis and follow-up of CS. 18F-FDG imaging is based on the fact that inflammatory cells, especially macrophages, use glucose in their metabolism. 18F-FDG is a glucose analog, which after being absorbed phosphorylates and thus cannot be released from the cells. Under aerobic circumstances, healthy myocardium utilizes mainly free fatty acids in its metabolism (145). As up-regulation of glucose metabolism occurs at sites of

macrophage-mediated inflammation, accumulation of 18F-FDG suggests an active inflammatory process (208-210). 18F-FDG-PET study is usually combined with myocardial perfusion scan to detect associated scarring (145,211). In theory, the 18F-FDG-PET findings can be divided into three phases according to the disease activity. In the early inflammatory stage, focal or patchy FDG accumulation prevails. In the more advanced stage a typical finding is a “mismatch” or “hot spot” with FDG accumulation superimposed upon a perfusion defect. At a late stage of CS, with permanent scarring but without ongoing inflammation, only a perfusion defect is detected (145).

The advantage of 18F-FDG-PET in diagnostics of CS is its high sensitivity, being 100% compared to JMHW 1993 guidelines in many studies (209,212). A systematic review of seven studies that evaluated the accuracy of 18F-FGD-PET with JMHW 1993 criteria yielded 89% sensitivity (208). The specificity of 18F-FDG-PET is lower, ranging from 38.5% to 90% and being 78% in the pooled analysis defined by the JMHW guidelines (193,208,212,213). A speculated explanation for this might be that 18F-FDG depicts early-stage sarcoid lesions before patients meet the diagnostic criteria (193). Many studies have used qualitative visual analysis of FDG uptake in the heart muscle. Recently, the introduction of quantitative assessment using standardized uptake values and FDG volume-intensity analysis has increased the accuracy of 18F-FDG-PET, thereby improving its diagnostic and prognostic value (210,213,214).

In addition to its diagnostic utility in CS detection, 18F-FDG-PET provides functional information of inflammatory activity and can thus help evaluate disease activity and treatment response (211-215). Like CMR, 18F-FDG-PET imaging also predicts adverse cardiac events (108,213) with a mismatch pattern (see above) and right ventricular involvement being particularly strong signals of high arrhythmia risk (213). Yet another benefit of 18F-FDG-PET imaging is the possibility to detect extracardiac inflammation and thereby targets for biopsies to verify sarcoidosis histology, most notably mediastinal lymph node biopsies (1,213,216) (**Figure 8**).

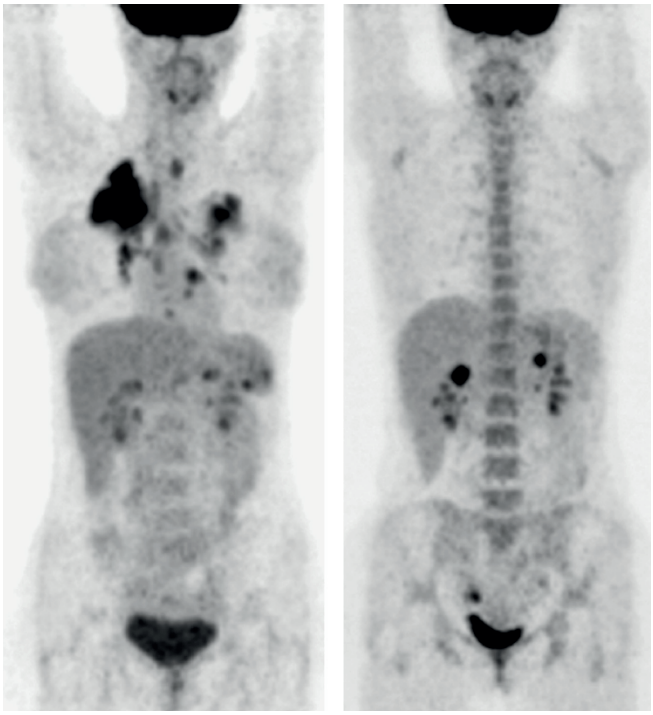


Figure 8. 18-F-FDG-PET whole-body images. 18-F-FDG uptake in mediastinal and hilar lymph nodes and lungs (Left panel) and no abnormal 18-F-FDG accumulation after 10 months of treatment (Right panel). Adapted from (216).

A proper technical imaging protocol is of primary importance in acquiring representative diagnostic data from 18F-FDG-PET (193,210). Since normal myocytes also utilize glucose in metabolism, it is necessary to suppress the physiological FDG uptake through a high-fat/low-carbohydrate diet, overnight fasting and/or administration of unfractioned heparin (to stimulate lipoprotein lipase which hydrolyzes triglycerides to free fatty acids and glycerol) (209,210,212,217).

Finally, 18F-FDG-PET and LGE-CMR can be utilized as complementary imaging methods (193). As discussed, 18F-FDG-PET is more sensitive and serves better at assessing early inflammatory phase whereas CMR in detecting even small areas of scars (1,26). The sensitivities and specificities of different imaging modalities vary, partly depending on which CS diagnostic criteria are applied (**Table 6**).

Table 6. Approximated sensitivities and specificities of imaging modalities for the detection of CS. Adapted from (110).

Imaging modality	Sensitivity	Specificity
Echocardiography	Low	Low
TI-201 or Tc-99m scintigraphy	Moderate	Moderate
Ga-67 scintigraphy	Low	High
18F-FDG-PET	High	Moderate to high
LGE-CMR	Moderate to high	High

CS= cardiac sarcoidosis, TI-201= thallium-201, Tc-99m= technetium-99m, Ga-67= gallium-67, 18F-FDG-PET= 18F-fluorodeoxyglucose positron emission tomography, LGE-CMR= late gadolinium enhancement cardiac magnetic resonance imaging.

2.5.2.5 Other imaging studies in CS

Various radionuclides, including thallium (TI-201), technetium-99m (Tc-99m) and gallium-67 (Ga-67) have been used in myocardial scintigraphies to evaluate CS (1,158,218,219). The typical abnormality in thallium-201 and technetium-99m myocardial perfusion imaging is "reverse distribution", i.e., the presence of a focal perfusion defect at rest that decreases or disappears under stress (220). The hypothesized explanation for this is focal reversible vasoconstriction at rest in arterioles adjacent to granulomas (1). Gallium-67-citrate is a radionuclide used for imaging inflammation that was previously widely utilized in lung sarcoidosis and in the detection of myocardial involvement and response to corticosteroids (221). Positive gallium-67 uptake is still included in the revised JMHW guidelines (178). Gallium-67 locates inflammatory areas of myocardium with high specificity (221,222). However, the reported sensitivity is lower and gallium-67 may miss fibrosis and dormant CS (219,223). Where available, 18F-FDG-PET and LGE-CMR have replaced the use of the other radionuclide imaging studies (1).

Thoracic computed tomography can identify mediastinal lymphadenopathy and thus help to differentiate CS from DCM. Otsuka et al. reported that 88% (7/8) of patients later diagnosed with CS, compared to 5% (1/20) of age-matched patients with idiopathic DCM, exhibited significant mediastinal lymph node swelling at computed

chest tomography (100). It is notable that, conventional chest X-rays did not show lymphadenopathy in any of the patients. Further, computed tomography can help to reveal sarcoid pulmonary and mediastinal involvement and to exclude coronary artery disease in young patients with low pretest probability (4).

2.5.2.6 Cardiac imaging in GCM

In acute GCM, a typical finding in echocardiography is reduced LVEF together with normal LV dimensions, which progresses to further LVEF deterioration and LV dilatation, sometimes over a matter of days (224,225). Other echocardiographic abnormalities in GCM may include aneurysms and wall thickness abnormalities, particularly thickening caused by extensive inflammatory cell infiltrate (226,227). Data on LGE-CMR in GCM is very limited because the largest GCM studies were conducted prior to the golden age of CMR, and many patients are too unstable to undergo the imaging in the acute phase (26). In case reports of severe GCM, CMR has shown multiple myocardial LGE areas in different myocardial layers, but the findings only partially correlated with histological examinations at autopsy or of explanted heart (228,229). In a population of 86 patients with suspected non-ischemic cardiomyopathy, more extensive LGE predicted CS and GCM (230). Finally, CMR is a widely studied and established imaging method in myocarditis, involving largely the same pathophysiologic mechanisms including edema, hyperemia, capillary leakage, necrosis and fibrosis as GCM (231-233). The data on 18F-FDG-PET in GCM is extremely limited (230).

2.5.2.7 Diagnostics biopsies

A definite diagnosis of CS and GCM requires a histologic confirmation from the heart (2,4). The histological hallmark of sarcoidosis is a non-caseating granuloma, as opposed to caseating granulomas in tuberculosis (5). The sarcoid granulomas are histologically distinct from interstitial rheumatic granulomas and vascular lesions in Wegener`s granulomatosis (27). Furthermore, other causes of granulomatous infiltration need to be excluded on special stains (tuberculosis on Ziehl-Neelsen and fungal infections on Gomorri methenamine silver). Sarcoid granuloma is a nodus containing mononuclear phagocytes (macrophages, epithelioid cells and giant cells) in the center, surrounded by T-lymphocytes, plasma cells and mast cells and circulated by a fibrous ring (3). The histologic criteria for GCM on the other hand, requires the presence of multinucleated giant cells, myocyte necrosis and lymphocytes in the absence of well-formed granulomas, although sporadic, partial granulomas may be observed (10). Besides necrosis, eosinophils are more common in GCM, while fibrosis, especially when widespread and well-organized, strongly favors the diagnosis of CS (5,10,105). Disregarding the sometimes problematic differential diagnostics between CS and GCM, EMB is highly specific in these diseases (10).

In young or middle-aged patients with 2nd or 3rd degree AV-block, new-onset heart failure or ventricular arrhythmias without ischemic heart disease, the current

guidelines recommend EMB (4,231,234). EMB is a class IB recommendation (recommended with intermediate evidence of usefulness) in new-onset (<2 weeks) heart failure with hemodynamic compromise (234). The same recommendation applies for heart failure of two weeks to three months in duration with dilated LV and new ventricular arrhythmias, or 2nd or 3rd degree AV-block, or failure to respond to normal care within one to two weeks (234). Furthermore, EMB is a class II recommendation (may be considered with conflicting evidence) in heart failure of >3 months or unexplained ventricular arrhythmias (231,234). These recommendations are based on incremental diagnostic, prognostic and therapeutic value, which histological confirmation of CS, GCM, eosinophilic or other treatable myocarditis signals (86,234).

Nevertheless, due to the heterogeneous, patchy distribution of the microscopic granulomas in myocardium, the diagnostic yield of EMB in CS is poor. Sarcoidosis classically involves the base of the heart, leaving standard mid-ventricular and apical biopsies unrevealing (235). Different studies have demonstrated EMB sensitivity of 15-30% in CS (2,21,86,91). The histologic diagnostic rate is dependent on the stage of CS and the timing of the biopsies. The success rate was 36.4% in a DCM-like clinical picture compared to 6.7% in cases with conduction disturbances and preserved systolic function (2). The study by Ardehali et al. suggested that a limited cardiac sarcoid involvement that was not detected by EMB, predicted better survival, though by no means freedom from cardiac death, compared to EMB positive cases (86). In GCM, at least in the case of fulminant disease, the yield of EMB is higher, with a sensitivity of 80-85% (236). A greater number of biopsies increases the diagnostic yield in myocarditis in general, but the value of multiple and/or repeated EMBs in CS/GCM was previously undetermined (237). Conventionally, EMBs are taken from the RV but in some cases, e.g., in imaging findings concentrating on the left side, LV biopsies may be usefull with reported complication rates similar to right-sided procedures (237,238).

Pre-procedural CMR and/or 18F-FDG-PET imaging is recommended and usually performed in clinical practice (231). Biopsies targetting areas with detected inflammation has raised interest, but there are no prior reports on its usefulness in CS/GCM and the utility in myocarditis in general is poorly defined (231,237). Novel methods to improve the diagnostic yield of EMBs are needed. Recently, electroanatomic mapping-guided EMB targeting low voltage and fragmented signal areas significantly improved the diagnostic yield in suspected myocarditis or CS (137,235). Furthermore, a recent pilot study utilizing three-dimensional electroanatomic mapping with the recording electrode placed at the tip of the bioptome showed promise for better diagnostic gain (239).

Finally, due to the limited sensitivity of EMB, histologic confirmation of sarcoidosis can be acquired from extracardiac organs in the case of compatible cardiac symptoms and signs. The recent expert consensus statement recommended extracardiac biopsy sites to be targeted first because of higher diagnostic yield and lower procedural risk (4,177). One small prior report demonstrated mediastinal lymphadenopathy at chest-computed

tomography in 88% (7/8) of CS patients compared to 5% (1/20) of DCM patients, but did not report lymph nodes as potential biopsy targets (100).

Gene expression profiling of EMB samples is a promising diagnostic tool for differentiating CS, GCM, active lymphocytic myocarditis and non-inflammatory myocardial diseases. To date, two small studies have demonstrated that genes encoding regulators of immune response, cellular receptors including Toll-like receptors, and proteins of the mitochondrial energy metabolism are differentially expressed in these conditions (60,104). This is an important target for future research.

2.5.3 Laboratory markers

Sarcoidosis presents with a weak systemic immunologic response despite the associated extensive local inflammation, and thus biomarkers measured from blood are not of notable clinical use (33). Granulomas produce angiotensin-converting enzyme and their serum levels are elevated in 60% of patients with sarcoidosis. Still, the diagnostic value of angiotensin-converting enzyme is controversial in systemic sarcoidosis, let alone detection of cardiac involvement (33). Circulating lysozyme or calcium concentrations are no more helpful. Elevated plasma N-terminal pro-B-type natriuretic propeptide (NT-proBNP), a marker of cardiac overload, has been observed in sarcoidosis patients with cardiac complications (240).

An early report found first-generation cardiac troponin-T measurements to be ineffective in CS (240). Instead, high-sensitivity cardiac troponin-T (hs-cTnT) measurements were recently shown to correlate with 18F-FDG-PET findings in CS, being abnormally elevated in most PET-positive patients and normal in the majority of PET-negative patients (241). One case report described a rapid decrease in high-sensitivity cardiac troponin-I (hs-cTnI) following intravenous methylprednisolone administration (242). The value of high-sensitivity troponins in the detection of myocyte injury and evaluation of disease activity remains to be solved. Galectin-3 is a promising biomarker involved in heart failure, inflammation and fibrosis that has been studied in a small cohort of six CS patients (179,243). Galectin-3 was found to be higher in pulmonary sarcoidosis patients compared to healthy controls, but did not differ between CS and non-CS groups (179,243).

2.6 Management

The treatment of CS involves immunosuppressive medication for sarcoidosis and cardiac-specific treatment for heart failure, conduction disturbances and arrhythmias. No international guidelines exist on the management of CS and the best available data is based on meta-analysis and expert consensus (197,244). In 2012 a consensus statement on the management of CS, based on responses from 42 commendable sarcoidosis

experts to a Delphi method questionnaire, was published in the U.S. (197). Although there were significant differences of opinion among the experts highlighting the difficulties in managing CS, sufficient agreement was reached to formulate a proposal for best practice, **Figure 9**.

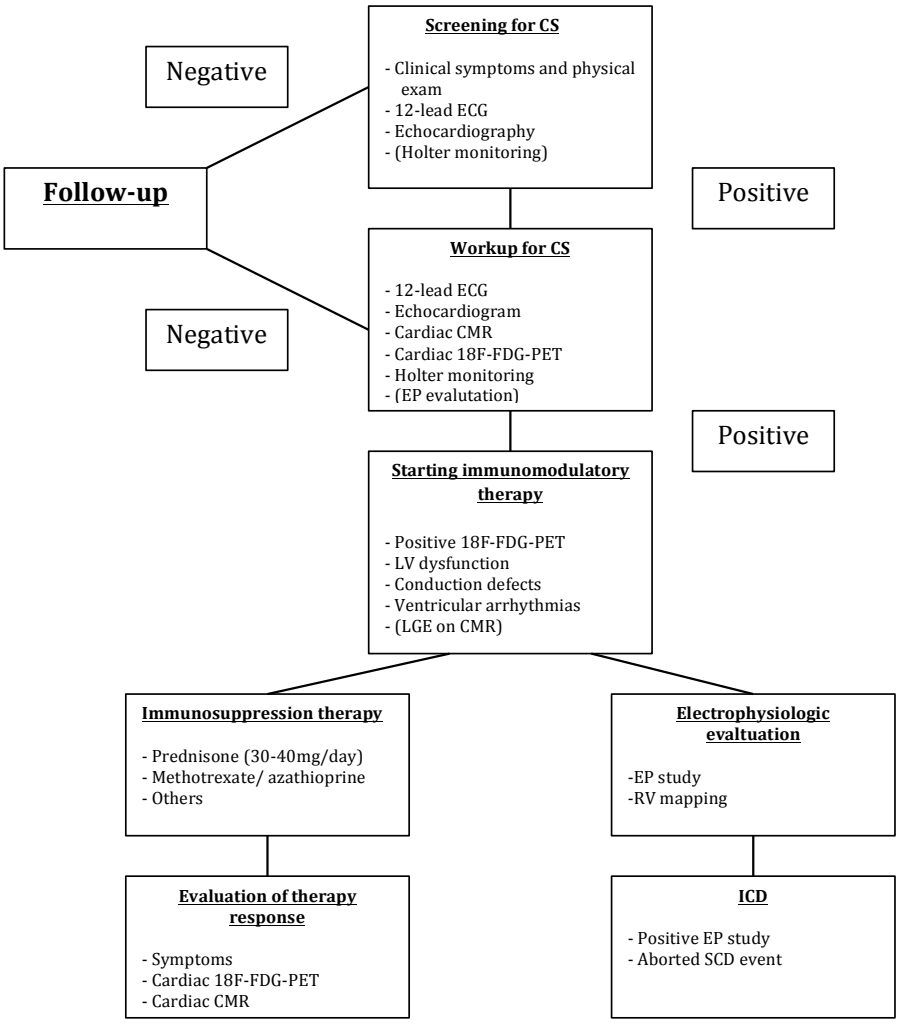


Figure 9. Proposed best practice for the diagnosis and treatment of CS based on expert panel consensus. Adapted from (197).

CS= cardiac sarcoidosis, ECG= electrocardiogram, CMR= cardiac magnetic resonance imaging, 18F-FDG-PET= 18F-fluorodeoxyglucose positron emission tomography, EP= electrophysiologic, LV=left ventricle, RV=right ventricle, SCD= sudden cardiac death.

2.6.1 Corticosteroids

2.6.1.1 Implementation

The goal of immunosuppressive treatment in CS is to reverse ongoing inflammation and thereby potentially prevent progression to fibrosis and adjacent organ dysfunction (197). As in sarcoidosis affecting other organs, corticosteroids are the mainstay of therapy for cardiac involvement. Corticosteroids function by suppressing multiple pro-inflammatory cytokines and chemokines (245). However, debate exists as to whether treatment can change the outcome of sarcoidosis in general and the evidence is even more incomplete in CS (29,43).

As there are no randomized controlled trials, the choice, dosage and duration of therapy still remain to be judged by clinicians. In general, prednisone starting dosage varies from 20-60mg/day or 50-60mg on alternate days. In the study by Yazaki et al. there was no difference in outcome in low (≤ 30mg) compared to high (≥40mg) initial prednisone daily dose (97). The dose of prednisone is gradually tapered down to a maintenance dose of 5-15mg/day (6,97,244). Controversy remains on the duration of the treatment (244). Some centers repeat 18F-FDG-PET scans or other cardiac imaging modalities to guide long-term steroid treatment(197,244). Finally, after stopping immunosuppressive therapy, close follow-up for possible future relapses is recommended by the experts (244).

2.6.1.2 Efficacy

Despite over 50 years of the use of corticosteroids in CS, there still is no proof of survival benefit from immunosuppressive treatment (43,244). In the largest study on long-term survival, the five-year survival rate was only 10% in patients not receiving steroids compared to 75% in steroid-treated patients, but since all the 20 non-treated patients were diagnosed at autopsy, no conclusion on the efficacy of steroid treatment could be drawn (113). The same dilemma applies to other studies as there are no placebo-controlled treatment trials. Kato et al. retrospectively evaluated the survival of seven patients receiving corticosteroids and 13 patients not receiving these agents and found that in the untreated group, two patients died compared to no deaths in the treatment group, but the difference was statistically insignificant (246). Likewise, Nagai et al. found no effect on cardiac deaths in the 7.6-year follow-up in 67 patients with corticosteroid treatment compared to 16 patients without corticosteroids (reasons for withholding corticosteroids; cardiac imaging without active inflammation in seven, patient refusal in five, active infectious disease in two and others in two) (115).

The data concerning the efficacy of corticosteroids on LV function is contradictory. In a cohort of 30 patients in a study by Takaya et al., LVEF remained mildly reduced yet stabile over 12 months of steroid treatment (215). Conversely, in a cohort of 10 patients from Kudoh et al., mean LVEF improved from 35% to 49% after six months of steroid therapy (247). In a study of 20 CS patients with AV-block and initially normal LVEF, seven corticosteroid-treated patients demonstrated no change in LVEF, compared to 11

out of 13 non-treated patients with marked decline in LVEF over a mean follow-up period of 79 months (246). Similarly, in a recent study by Nagai et al., patients with corticosteroid therapy (n=67, initial mean LVEF 36%), had an increase in LVEF (+8% ±36%) versus decrease in LVEF (-17 ±35%) in non-steroid group (n=16, initial mean LVEF 35%) (115). Furthermore, the difference in LVEF was emphasized in patients with baseline LVEF ≥35% and steroid-treated patients also had fewer hospital admissions due to heart failure (115). Furthermore, Chiu et al. demonstrated no effect on LVEF either in the total group of 43 CS patients or in patients with severely reduced LVEF (<30%, n=11), but a significant improvement in LVEF and LV end-diastolic diameter (LVEDD) in the group with mildly to moderately decreased LVEF (30-54%, n=10) (6). A systematic review summarizing four studies (not including the recent study from Nagai et al.) concluded that steroid therapy seems to maintain LV function in patients with normal LVEF at diagnosis, improve LVEF in patients with mild to moderate dysfunction, but have no effect in patients with severe dysfunction (244). Anyhow, the authors underline that it is unclear whether the improvements were due to corticosteroids or were simply indicative of the natural course of the disease (244).

Several small patient series have shown that AV-conduction blocks mainly develop during the inflammatory phase of CS and that recovery of conduction is possible with corticosteroid treatment (124,246,248). In the study by Yodogawa et al. (2013) (248), AV-block resolved within the first week of steroid therapy in four of seven patients and later (up to 14 months) in the remaining three patients out of a total of 15 patients. The restoration of conduction was more common in patients with preserved LVEF (248).

In studies with CS patients having ventricular arrhythmias at presentation, initiating steroid treatment has shown no beneficial effect on the arrhythmia load (124,132). However, in a small study of patients without presenting ventricular arrhythmias, 14.3% (1/7) of patients treated with steroids compared to 61.5% (8/13) of non-steroid-treated patients had ventricular arrhythmias during the disease course (246). The study by Yodogawa et al. (2011) showed a significant decrease in VPBs and non-sustained VTs, as well as an improvement in signal-averaged ECG parameters in patients with EF ≥ 35%, but not in patients with EF < 35% over a seven-month period of corticosteroid therapy (249). In 45 CS patients with ICD, post-implantation immunosuppression had no effect on the ventricular arrhythmias (128).

Several 18F-FDG-PET studies have demonstrated the diminution or disappearance of 18F-FDG uptake in CS after one to two months' steroid therapy (211,214,215). Likewise, in a scintigraphy study, corticosteroids abolished gallium-67 uptake within six months in all nine patients with positive uptake at presentation (124). In a CMR study of 12 histologically verified patients with systemic sarcoidoisis and suspected CS based on scintigraphy results, six (including three with cardiac symptoms) received corticosteroids with CMR showing cleared or improved findings in all at the 12-month follow-up, compared to a worsening or stability of CMR findings (signal intensity, contractility and myocardial thickness) in the six non-treated patients (206). Whether

the reduction of myocardial inflammation measured by imaging findings is related to clinical improvement is controversial. Osborne et al. found a significant inverse relationship with LVEF and standardized uptake values in PET (214), whereas Takaya et al. demonstrated resolution of 18F-FDG and gallium-67 scintigraphy activity without any improvement in LVEF (215). Altogether, there is evidence from relatively small studies that the corticosteroid treatment response in CS might be positive in the early inflammatory phase of disease but no longer when myocardial fibrosis and scars have formed (6,124,215,249).

2.6.2 Other immunosuppressants

Long-term use of steroids is associated with adverse effects (diabetes, cataract, osteoporosis, weight gain, gastrointestinal bleeding, infections, etc.), and alternative agents are also required for patients refractory to corticosteroids. No randomized comparative studies of steroid-sparing immunosuppressants in CS exist. In a study of 17 patients, a combination of methotrexate (6mg/week) and a low-dose (5-15 mg/day) of prednisolone showed a tendency towards better outcomes compared to corticosteroids alone (with no information of maintenance dose) measured by higher LVEF, smaller LVEDD and lower NT-proBNP over five years of follow-up (250). In a Delphi study collecting the opinions of sarcoidosis experts, methotrexate was the second most commonly used drug, followed by azathioprine, mycophenolate mofetil, hydroxycholoquine, and anti-TNF-α agents (88,197). There are case reports showing that anti-TNF-α agent infliximab may be effective in CS (251,252). The use of infliximab is theoretically supported by the well-known role of TNF-α in granuloma formation (32,41,42). However, infliximab has been shown to worsen the outcome in severe systolic heart failure in general (253), and it should therefore be used with great caution, particularly with advanced CS.

2.6.3 Management of heart failure and arrhythmias

Besides immunosuppression, the treatment of CS includes management of ventricular dysfunction, heart failure, and cardiac rhythm disturbances (88). Ventricuar dysfunction is managed according to the established guidelines for heart failure with beta blockers, angiotensin-converting enzyme inhibitors, and mineralocorticoid-receptor antagonists (254). The treatment of ventricular arrhythmias is based on both drugs and devices (see below). In a multicenter study of 235 patients, the most frequently used antiarrhythmic agents, in addition to beta blockers, were sotalol and amiodarone (129). Class I (Vaughan-Williams) antiarrhythmic drugs should be avoided in ventricular and atrial arrhythmias alike due to their potential harmfulness in structural heart disease (255).

2.6.4 Device therapy and catheter ablation

Since ventricular arrhythmias in CS usually recur, ICD therapy has long been recommended for patients with sustained VT or VF (132). More recently, ICDs have been increasingly implanted for primary prevention, especially in patients with non-sustained arrhythmias on ambulatory ECG, unexplained or near syncope, or positive electrophysiological study in the setting of reduced EF (90,197). In large studies recently published in the U.S., 63-74% of ICDs in CS patients were implanted for primary prevention in patients without prior significant ventricular arrhythmias (98,128,129). Besides a high rate of appropriate ICD therapies (see Arrhythmias section), CS patients are also reported to experience inappropriate shocks (13.3-25%)(98,128,129) and device-related complications (15.6-18%) relatively commonly (99,128,129). In the general ACC/AHA/HRS 2013 guidelines for cardiac pacing, CS was classified as IIA, level of evidence C indication for ICD, meaning that implantation should be considered (256). In the 2013 ESC/EHRA guidelines CS was not separately addressed (257). An expert consensus statement on CS recommends ICD for all CS patients with spontaneous sustained VTs and/or LVEF ≤ 35%, despite optimal medical therapy and a period of immunosuppression (if there is active inflammation) (4). Independent of LVEF, ICD implantation can be useful in cases of indication for bradycardia pacemaker, unexplained syncope or near syncope or positive electrophysiological studies (4). In case of mildly to moderately reduced LVEF (36-49%) and/or RVEF <40%, ICD implantation may be considered (4). If none of the aforementioned symptoms or findings are present and there is no LGE on CMR, ICD implantation is not recommended, but the patients should be closely monitored (4).

As the disease-specific data on CRT in CS is minimal, general recommendations should be applied (4,257,258). In one Japanese study, CS patients (n=25) seemed to respond less to CRT compared to other non-ischemic cardiomyopathy patients (148) but this has not been confirmed. CRT is recommended in patients with sinus rhythm, LBBB with QRS duration ≥ 120ms, LVEF ≤ 35% and NYHA class ≥ II despite adequate medical treatment and should/may be considered in non-LBBB patients with the other aforementioned features (257). In patients with conventional pacemakers, upgrading to CRT should be done according to the aforementioned indications (257). The evidence in patients with conventional indication for anti-bradycardia pacing is less clear, but suggests that patients with moderate-to-severe LV dysfunction might benefit from CRT compared to RV pacing alone (257).

In the case of ventricular arrhythmias refractory to drug treatment or frequent adequate ICD therapies, radiofrequency catheter ablation is an option. In a survey study from 2012, 14.9% of 235 patients with ICD implantation for CS in major electrophysiology centers in the U.S., Canada, and India, underwent VT ablation (129). In two cohorts of patients with multiple antiarrhythmic medications without proper arrhythmia control, catheter ablation resulted in freedom of VTs in four out of eight patients (121) and either reduced or completely eliminated VTs in four and five out of

nine patients, respectively (122). In a study of 21 patients who were refractory to medical treatment, VT ablation was successful in terminating ≥ 1 spontaneous VT in 90% and in eliminating electrical storm in 78% of patients (126). However, probably due to multiple arrhythmia substrates, VT recurred in all but three patients after a single procedure, and repeated procedures were needed to achieve better arrhythmia control with fewer antiarrhythmic drugs (126). Altogether, catheter ablation appears to be effective in treating VT storm in CS and may provide palliation for recurring arrhythmias, but due to diffuse myocardial involvement, achieving full arrhythmia control is difficult (126). Data concerning ablation of atrial tachyarrhythmias in CS is limited to one study that showed lasting results in seven of nine patients during a mean follow-up period of 1.8 years (140).

2.6.5 Transplantation

Cardiac transplantation is a viable treatment in end-stage heart failure or uncontrollable ventricular arrhythmias when other organs are not significantly damaged. CS accounts for up to 2.5% of all cardiac transplantations (82-84,173,259).

Sarcoidosis has been reported to recur in the transplanted heart with a rate ranging from 0% (0/19) to 14.3% (2/14) in approximately five years (82,173). A recurrence can follow the tapering of corticosteroids (260) and is often treatable with an elevated corticosteroid dose (82,173,261).

The data concerning prognosis after cardiac transplantation in CS is somewhat contradictory. The largest multicenter study of 38,230 transplantations with 65 CS patients demonstrated slightly better one-year survival in CS patients compared to patients transplanted for other cardiac conditions (259). Conversely, a single-center study of 825 transplantations including 14 CS patients showed a trend towards higher mortality in CS patients with a five-year post-transplantation survival rate of 58.9% compared to 76.2% (82). In the latest study no survival difference was observed, with five-year post-transplantation survival being 79% in 19 CS patients compared to 83% in 1,050 other heart transplant recipients (173). A case of possible transmission of sarcoidosis from a donor to a recipient has been described (262).

2.6.6 Management of GCM

The only trial designed to evaluate immunosuppressive treatment response with placebo in GCM, by Cooper et al., failed to recruit patients in the non-immunosuppression arm, making the estimation of survival benefit impossible (107). Nevertheless, the study design was modified to examine the effect of immunosuppressive therapy as such and recruited 11 patients from 17 medical centers around the world. EMBs controlled at four weeks after the start of combined immunosuppressive treatment showed significant a reduction in the amount of

inflammatory cells and necrosis. Anyhow, LVEF did not change significantly after a month of treatment, presumably because of relatively mild cardiac dysfunction at the start of the study (mean 44±18%). At the follow-up after one year, two patients required heart transplantation and one patient died of recurrent GCM after stopping all immunosuppressive medication. In addition to the study by Cooper et al., a small study of five GCM patients also found improvement in histological findings but no effect on LVEF after six months of immunosuppressive therapy (9). Recently, a stepwise histopathologic improvement with multiple immunosuppressants was documented, from fulminant myocarditis with giant cells to a myocarditis characterized by a lymphocytic-eosinophilic infiltrate, further to a smoldering lymphocytic myocarditis and lastly to interstitial (mostly replacement-type) fibrosis in repeated EMBs (166). However, in the same study the degree of immunosuppression during the first year after diagnosis did not predict the subsequent composite endpoint event of death, transplantation, first ventricular arrhythmia or GCM recurrence (166). Furthermore, several case reports have demonstrated dramatic clinical and histological recoveries from GCM cardiogenic shock treated by immunosuppression (69,263-265). Finally, experimental murine autoimmune myocarditis studies indicate a benefit from T-cell suppression therapy in GCM (62).

No international guidelines on therapy of GCM exist, and the studies addressing its treatment are small. The immunosuppression protocol in the GCM treatment landmark study involving 11 patients was as follows (107). Intravenous methylprednisolone 10mg/kg was administered for three days, followed by prednisone 1mg/kg with rapid tapering up to a year. Additionally, cyclosporine with serum target levels of 150-300ng/ml was used. Eighty-two percent of the patients received muromonab-CD3 (OKT-3), a monoclonal antibody against CD3-T-cells. In the up-to-date report of 26 GCM patients from Germany and the U.S., the majority of patients received cyclosporine-based multiple immunosuppression after diagnosis, including muromonab-CD 3 or a comparable antilymphocytic agent in 42% (166). Of them, 27% were weaned onto corticosteroid-only therapy during the first year and 73% continued with combination treatment (166). At any point in time, 26 patients (100%) received corticosteroids, 21 (81%) cyclosporine, 10 (38%) mycophenolate mofetil, eight (31%) azathioprine, and eight (31%) sirolimus. Furthermore, there are case reports of tacrolimus, anti-thymocyte globulin and alemtuzumab (a monoclonal CD52 T-cell surface protein antagonist) being administered with success in GCM (26,69,172,265,266). In addition to immunosuppressants, the treatment of GCM encompasses heart failure and arrhythmia-targeted therapies and, in patients with severe LV dysfunction, inotropic agents.

Despite the moderate success of intensive immunosuppression, a significant proportion of GCM patients require a heart transplantation, sometimes urgently (7,37,267). As a bridge to cardiac transplantation, mechanical circulatory support with ventricular assist devices, intra-aortic balloon pumps and extracorporeal membrane oxygenation have been used successfully in GCM (26,37,268). In the GCM landmark study, 54% (34/63) of

patients underwent transplantation with a median of six months after symptom onset. On follow-up after 3.7 years, 26% (9/34) of transplanted patients died (11). Post-transplant recurrence of GCM is more common compared to CS, with 26-43% (9/34 – 3/7) of transplanted patients having GCM findings in surveillance biopsies an average of three years (ranging from three weeks to nine years) after transplantation (11,37). Asymptomatic recurrence does not necessarily carry a poor prognosis and usually resolves with temporarily augmented immunosuppression (37). In the study by Cooper et al. (1997) six of the nine recurrences were asymptomatic and one died of heart failure (11).

2.7 Prognosis

2.7.1 Prognosis of CS

The survival data concerning CS is heterogeneous, depending on the diagnostic criteria used, the inclusion or exclusion of autopsy and post-transplantation diagnoses, and the reported survival definition reported (overall-, cardiac- or transplant-free cardiac survival) (**Table 7**). Some kind of treatment with a possible effect on the disease course has been used since the early studies, thus there are no reports strictly on the natural history of CS. In the first large series of 250 CS patients, in which the treatment was variable but steroids were commonly used, the five-year survival rate was 40% (22). In an early Japanese study, 55% (23/42) of CS patients died within one year from the onset of the cardiac symptoms (16). The most widely cited survival rates are from two series from the beginning of the 21st century, with five-year survival rates of 60-75% (5,97). Regarding the consequence of cardiac involvement in systemic sarcoidosis, CS was reported to cause two-thirds of deaths in Japanese sarcoidosis patients, but less than 20% of deaths in Caucasians (49,269).

Table 7. Prognosis of CS in different studies.

Study, year	N	Survival	Comments
Chiu et al. 2005 (6)	43	Overall Lifetime diagnosis (N=43) 1 year 98% 5 years 90% 10 years 84%	No cardiac deaths in patients with normal LVEF at diagnosis

Okura et al. 2003 (5)	42	Transplant-free Lifetime/autopsy/ explant diagnosis (N=42) 5 years 61% Lifetime diagnosis (N=30) 5 years 70%	Survival significantly better compared to GCM
Yazaki et al. 2001 (97)	95	Overall Lifetime/autopsy diagnosis (N= 95) 1 year 85% 5 years 60% 10 years 44% Lifetime diagnosis (N=75) 1 year 92% 5 years 75% 10 years 61%	Overall survival in patients with lifetime diagnosis and LVEF ≥ 50%; 1 year 95%, 5 years 89% and 10 years 89%
Fleming et al 1986 (22)	250	Overall Autopsy diagnosis 5 years 40% 10 years 14%	Steroid used commonly, otherwise treatment data nonexistent
Roberts et al. 1977 (109)	113	Overall Autopsy diagnosis 1 year 27%	
Matsui et al. 1976 (16)	42	Overall Mostly autopsy diagnoses* 1 year 45% 2 years 26%	*Only 5 (12%) lifetime diagnoses

CS= cardiac sarcoidosis, LVEF= left ventricle ejection fraction, GCM= giant cell myocarditis.

All the aforementioned studies are retrospective. Subsequently, several small studies on patients with systemic sarcoidosis, diagnosed with CS and monitored prospectively since, have shown comparable survival rates. Among 101 pulmonary sarcoidosis patients from the Netherlands, 16 patients were diagnosed with symptomatic CS according to the modified JMWH criteria, and 25% (4/16) of them died over a mean follow-up of 1.7 years (77). Similar results were reported from the U.S., with 19% (4/21) of patients with biopsy-proven extracardiac sarcoidosis and LGE-CMR findings compatible with CS suffering cardiac death in the mean follow-up of 1.8 years (91). In contrast, in the study by Mehta et al., none of the 24 patients with pulmonary sarcoidosis and mostly asymptomatic CS diagnosed based on LGE-CMR and 18F-FDG-PET imaging died during a mean follow-up period of 1.8 years (93). One might speculate that the outcome with modern heart failure treatment and prophylactic use of ICDs should be better. Unfortunately, the recent large ICD follow-up studies have not reported survival numbers (98,129). In the latest study with survival data, 15% (7/47) of patients with CS diagnosed according to the JMHW 2006 guidelines died over a median follow-up period of 15 months (270).

LV systolic function is the most powerful predictor of outcome in CS. In a study of 95 patients, 10-year survival was 89% in patients with preserved EF (≥ 50%) compared to 27% in patients with reduced EF (<50%) (97). In another study of 43 patients, 10-year cardiac survival was 100% in patients with normal EF (≥ 55%) but only 19% in those with severely reduced EF (<30%) at the start of the follow-up (6). Other predictors of survival in CS include LV end-diastolic diameter, New York Heart Association (NYHA) functional class, and sustained VT (97). Interestingly, the presence of pulmonary involvement has been associated with better survival, probably due to earlier detection of cardiac involvement during the follow-up of pulmonary lesions (97). Eventually, in recent years LGE in CMR has been suggested as an independent predictor of future adverse cardiac events in CS (202-204) (see 2.5.2.3).

2.7.2 Prognosis of GCM

The course of GCM is often fulminant and its prognosis is therefore in general poorer than the prognosis of CS. In an early study of five EMB-verified patients, 60% (three patients) had died or undergone transplantation during a follow-up period of 1.7 years (9). In the multicenter landmark study of 63 patients with GCM, the rate of death or transplantation was 89% and median transplant-free survival from symptom onset in all patients was 5.5 months (11). Notably, a total of 25 patients (39%) were identified and included only after autopsy or from explanted hearts. Combined immunosuppressive treatment including cyclosporine together with another immunosuppressant, but not corticosteroids alone, prolonged the median survival from 3.0 (without any immunosuppressants) to 12.3 months. However, the finding was biased resulting from the selection of survivors, i.e., those who would have survived longer even without immunosuppressive treatment were more likely to be treated with immunosuppressive therapy. A later study with a longer follow-up from the same group

demonstrated a 10% transplant-free survival rate after five years from symptom onset in all 73 patients and 22% five-year transplant-free survival in the 38 patients diagnosed before death or transplantation and treated with at least corticosteroids (5). Recently, Maleszewski et al. examined the long-term outcome in 26 GCM patients who survived the first year following diagnosis without transplant (166). Over the follow-up period of 5.5 years starting one year after diagnosis, 31% of patients either died (n=3) or underwent transplantation (n=5). The factors leading to fulminant progress and poor prognosis or indolent course in GCM are unknown.

3 AIMS OF THE STUDY

This study was designed to assess the epidemiology, characteristics, diagnostics and outcome of CS and GCM in Finland. More specifically the goals were the following:

1. To evaluate the detection rate and prevalence of CS since the turn of the 1990s (I).
2. To elucidate the role of CS and GCM in the etiologic spectrum of AV-block in young and middle-aged adults (III).
3. To assess the biopsy diagnostics of CS clinically isolated in the heart (II).
4. To examine the clinical manifestations of CS (I).
5. To determine the prognosis of CS with modern treatment (I).
6. To assess the clinical utility of hs-cTnT/I in CS (IV).
7. To evaluate the characteristics and outcomes of GCM with modern treatment (V)

4 MATERIALS AND METHODS

This work is essentially a retrospective study where the data generated during routine examinations, treatment, and follow-up of patients with CS and GCM were collected afterwards for research purposes. The project was launched by the real life clinical observation that there was a remarkable increase in the amount of patients diagnosed with CS, formerly considered an extremely rare myocardial disease, in our institute. The study was planned and initiated at Helsinki University Hospital (HUH) and further conducted in collaboration with the other Finnish university and central hospitals treating patients with CS and GCM. During this project, the Myocardial Diseases in Finland (MIDFIN)-study group was established, consisting of academic cardiologists at the five Finnish university hospitals. We also founded an internet-based national registry of CS and GCM to help with the collection and storage of data for future research.

4.1 Study population

Patients diagnosed with biopsy-proven CS (n=229) and GCM (n=49) in Finland between October 1988 and July 2015 were enlisted through the following process. In 2009, all five Finnish university hospitals and 17 central hospitals covering the whole of Finland, were contacted by email and if necessary by telephone. Possible patients were screened from hospital discharge data registries using the ICD-10 code I41.8*D86.8 (sarcoidosis of heart) and the code D86 (sarcoidosis) combined with one of the codes I42 (cardiomyopathy), I44.1-2 (2nd or 3rd degree AV-block), I45.3 (trifascicular block), I46 (cardiac arrest), I47.2 (VT), I49.0 (VF), I49.3 (premature depolarization), I50 (heart failure) or R00.1 (bradycardia). In addition, colleagues in different hospitals treating cardiomyopathies were asked to gather patients with CS and GCM in their care. The investigator (RK) personally visited each hospital with potential adult (>18 years) CS patients and scrutinized the screening data. The GCM patients were identified from HUH hospital registries.

4.1.1 Diagnostic criteria

For inclusion, a histologic confirmation of sarcoidosis was required and thus patients with clinically diagnosed CS were excluded. Histological analysis of the heart (EMB, cardiac tissue sample taken during open heart surgery or heart transplantation or at autopsy) was the golden standard. The histological criteria for sarcoidosis were the presence of at least one non-necrotizing epithelioid cell granuloma with no more than solitary giant cells and eosinophils and without myocardial necrosis and special stainings to rule out other causes of granulomatous inflammation if considered pertinent. The histological diagnosis of GCM required widespread inflammatory

infiltrate including multinucleated giant cells, eosinophlis, lymphocytes and histiocytes together with a variable degree of myocardial necrosis in the absence of well-formed granulomas. The histological tissue samples were originally analyzed by pathologists from different university hospitals. For study V, the GCM biopsy samples were reanalyzed by two experienced cardiac pathologists in HUH. If well-formed granulomas were detected, the patients were excluded for having CS according to the Multicenter GCM Study Group criteria (11).

In case of missing cardiac tissue verification, extracardiac organ histologic proof of sarcoidosis in combination with both clinical manifestation indicative of myocardial disease and abnormalities compatible with CS in either LGE-CMR, 18F-FDG-PET or echocardiography was required. The typical imaging findings compatible with CS were LGE in a non-coronary artery disease pattern in LGE-CMR, focal 18F-FDG uptake with or without a concomitant perfusion defect in PET, and LV dysfunction and/or septal abnormalities at echocardiography. In total, our inclusion criteria adhered to the recent expert consensus guidelines (4).

Isolated CS was defined as sarcoidosis involving the heart without prior history or evident signs or symptoms of sarcoidosis outside the heart. The diagnostic work-up for screening extracardiac organs included chest X-ray, clinical examination, basic laboratory exams, and a detailed medical history, but further studies such as chest-computed tomography or ophthalmologic examinations were not routinely conducted. Patients who had 18F-FGD uptake in mediastinal lymph nodes in PET but no signs of sarcoidosis elsewhere outside the heart were included in the isolated CS group.

4.1.2 Studies I-V

-The cohort in study I constituted of the 110 patients diagnosed with histologically proven CS across the whole of Finland between October 1988 and February 2012 detected through the aforementioned conduct. All the patients in studies II-III and some of the patients in study IV, were subgroups of this cohort.

-The cohort in study II (conducted prior to study I) was screened from HUH medical records and the EMB registry. Between January 2000 and December 2010, 576 patients underwent diagnostic EMBs, excluding transplant surveillance biopsies. Biopsy-proven CS was diagnosed in 52 patients and 33 of them without extracardiac manifestations were included in study II.

-The cohort in study III (conducted prior to study I) was identified from the HUH pacemaker registry. Between January 1999 and April 2009 there was a total of 6420 pacemaker implantations, of which 133 were for 2nd or 3rd AV-block in patients aged 18-55 years, and these patients constituted the population in study III.

-The cohort in study IV consisted of 62 patients from six Finnish hospitals with new-onset treatment naïve CS diagnosed between December 2010 and December 2014 with hs-cTnT/I measured before and following the initiation of corticosteroid treatment. To be included, the patients had to have estimated glomerular filtration >60 ml/min/1.73m2 by the Modification of Diet in Renal Disease (MDRD) formula.

-The cohort in study V consisted of the 32 patients diagnosed with histologically confirmed GCM and seen in HUH between January 1991 and December 2011. The patients were identified from HUH medical records and HUH pathology registry.

4.2 Data collection

After patient inclusion, RK reviewed the relevant hospital charts for data on patients' demographics, histologic findings, symptoms, initial and later clinical manifestations, results of imaging and laboratory studies, evolution of LVEF, invasive procedures, details of treatment with drugs and devices, and the occurrence of adverse outcome events. Since the initial data collection on-site in different hospitals, the follow-up information on disease progression and adverse cardiac events was collected through the MIDFIN network. The last CS patients were included in February 2012 and adverse events were recorded up to the end of December 2013 (I). For this thesis, the number of new CS and GCM cases was updated until July 2015 and the updated data is in part unpublished. The work started in 2008 and data collection was fully retrospective between 1988 and 2008 and partly prospective thereafter. The causes of death were determined by hospital chart review. The mortality data was double-checked from the Finnish Population Register Centre in January 2014. All imaging studies were performed as part of routine clinical care. The measurements of biomarkers were taken and analyzed as part of clinical routine except some of the hs-cTnT measurements taken during outpatient visits at HUH (Study IV). Detailed imaging and biomarker analysis protocols are described in the original articles.

4.3 Ethical aspects

The nationwide study (I) had the approval of the national ethical review board (STM/1219/2009). The other studies (II-V) were approved by the institutional review board (Ethics Committee, Department of Medicine, Helsinki University Hospital). The studies were conducted according to the Declaration of Helsinki. The patients gave their informed, written consent to data collection for the national MIDFIN registry.

4.4 Statistical analyses

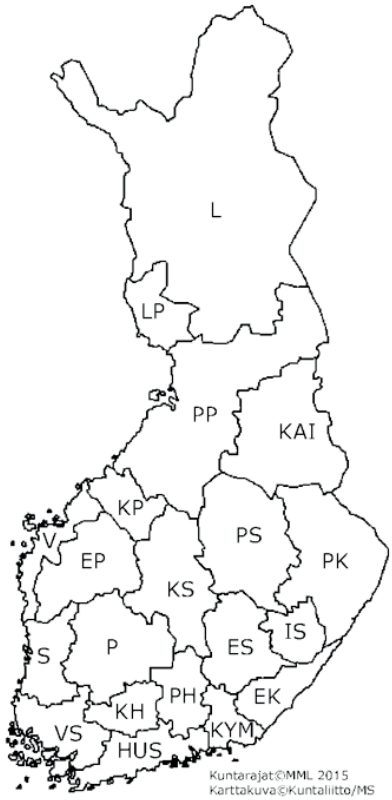
Continuous variables were presented as mean value ± SD when sample distribution was symmetric, and median (min - max) when sample distribution was skewed. Categorical variables were presented as absolute numbers and percentages. Comparisons between groups were analyzed using the Chi-square test, or Fisher's exact test when expected values were less than 5, for categorical data. For continuous variables, the Student's t-test, Kruskall-Wallis, analysis of variance or Mann-Whitney test were applied as appropriate. Within-group comparisons were made using Wilcoxon's signed ranks test and McNemar's test. In all tests, a 2-tailed $P<0.05$ was considered statistically significant.

Survival analyzes were calculated, first, from the onset of symptoms and, second, from the date of CS or GCM diagnosis. Thus, analyses were performed for total population and separately after excluding the cases diagnosed post-mortem or from explanted hearts. In study I the outcome events were presented separately for 1) cardiac death, 2) a composite of cardiac death and transplantation, whichever came first, and 3) a composite of cardiac death, transplantation, and aborted sudden cardiac death. In studies II-V the time-dependent outcome events included 1) a composite of cardiac death and cardiac transplantation (V), 2) aborted sudden cardiac death (VF treated by external or internal defibrillation), and sustained VT (II, III), and 3) new high-grade AV-block, whichever came first (IV). Survival curves free of the composite end point were plotted by the Kaplan-Meier method, and factors influencing survival were analyzed by the log rank test and by Cox regression analysis. The analyses were performed using SPSS versions 17.0-22.0 for Windows (SPSS Inc.; Chicago, IL, USA).

5 RESULTS

5.1 Incidence and prevalence of clinical CS and GCM in Finland (I,V)

A total of 229 CS patients were diagnosed in Finland from October 1988 through July 2015 (studies I-IV and Kandolin et al. unpublished results). The annual detection rate of CS was 0.6 per 100 000 adults (>18 years) in the last full 2-year study period between 2013 and 2014. The geographical distribution of CS patients in Finland is presented in **Figure 10**. The prevalence of histologically diagnosed CS cases in 2012 was 2.2 per 100 000 (I).



HUS 126
PPSHP 34
PSSHP 19
PSHP 17
PKSHP 10
VSSHP 8
KSSHP 3
EPSHP 2
ISSHP 2
KPSHP 2
SAT(=S)SHP 2
EKSHP 1
KHSHP 1
LSHP1
PHSHP1

_____

=229

Figure 10. Distribution of histologically diagnosed CS patients in Finland between 1988 and 2015 by health care districts.

SHP= sairaanhoitopiiri (Finnish for health care district), HUS= Helsingin ja uudenmaan, PP= Pohjois-Pohjanmaan, PS= Pohjois-Savon, P= Pirkanmaan, PK= Pohjois-Karjalan, VS= Varsinais-Suomen, KS= Keski-Suomen, EP= Etelä-Pohjanmaan, IS= Itä-Savon, KP= Keski-Pohjanmaan, SAT= Satakunnan, EK= Etelä-Karjalan, KH= Kanta-Hämeen, L= Lapin, PH= Päijät-Hämeen.

Over the study period of 26 years, the detection rate of CS multiplied. More specifically, the annual nationwide detection rate of 0.5 patients per year in the earliest two-year period between 1988-1989 increased to 27 per year in the last follow-up two-year period from 2013-2014 (**Figure 11**). Thus the number of patients diagnosed with CS increased by more than 50-fold during the study period.

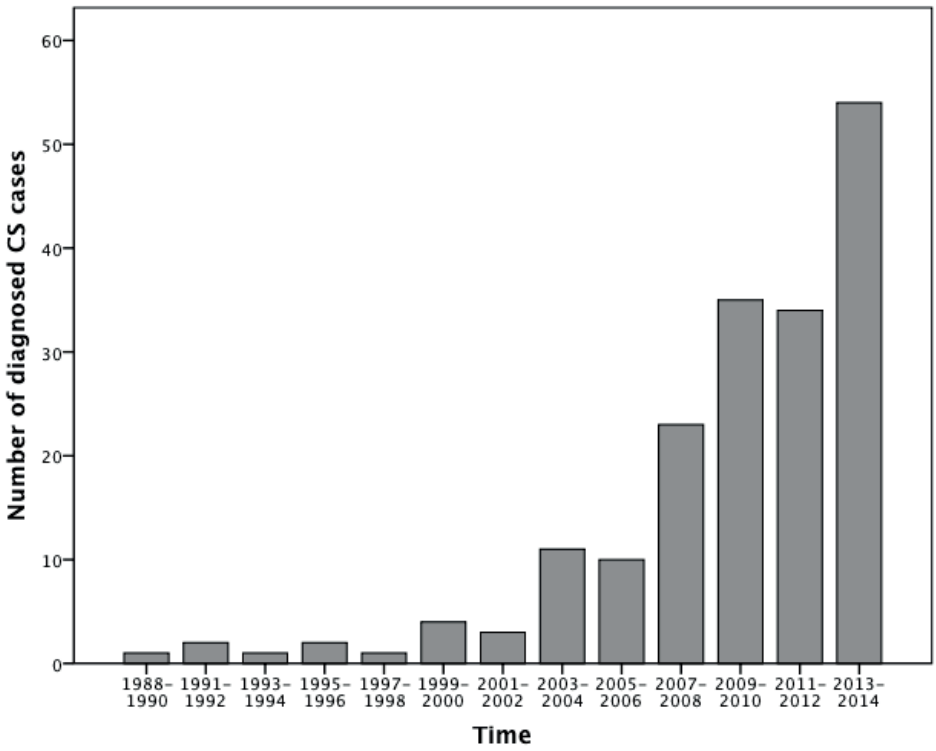


Figure 11. The number of new CS cases diagnosed in two-year periods from 1988 to 2014 in Finland.

A total of 49 patients with histologically confirmed GCM were seen at HUH during the GCM study period between June 1991 and December 2014 (study V and Kandolin et al. unpublished results). HUH is a nationwide referral center for cardiac transplantation and thus these GCM cases are likely to present the majority of GCM cases diagnosed in Finland over the 23-year range. Therefore, the annual detection rate of histologically verified GCM in Finland between 2013 and 2014 was 0.13 per 100 000. Similarly to CS, the diagnostic rate markedly increased over the study period, especially over the latter half of the study (**Figure 12**).

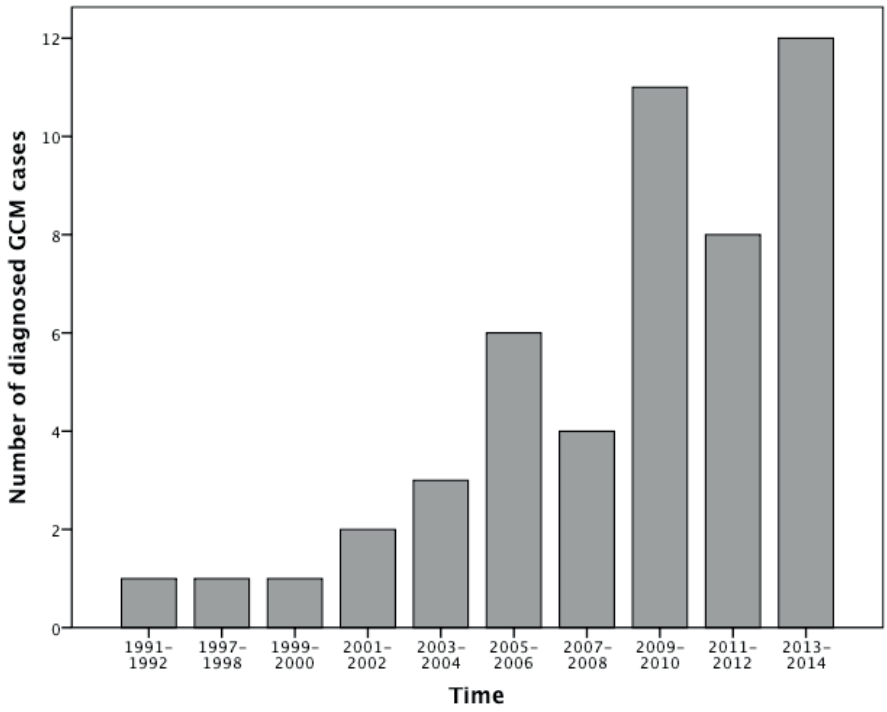


Figure 12. The number of new GCM cases diagnosed in two-year periods from 1991 to 2014 in Finland.

5.2 Patient characteristics and associated disorders (I)

5.2.1 CS

The mean age of the 110 CS patients was 51± 9 years, ranging from 27 to 69 years. There was a female predominance: 65% (71 patients) were women. **Table 8** presents the associated illnesses in CS patients (Kandolin et al., unpublished results)**.** CS was associated with other autoimmune diseases in 16% of cases (18 patients). The autoimmune disorders preceded the onset of CS in all cases. Thyroid disorders were most common in 12% (13 patients) and were overrepresented compared to the general population (271).

Seventy-one (65%) of the 110 patients with CS underwent coronary angiography or coronary computed tomography. Coronary artery disease was diagnosed in five (4.5%) patients. Two patients had mild coronary stenoses and were treated with pharmacotherapy alone. One patient underwent a percutaneous coronary intervention. Furthermore, one patient who underwent several percutaneous coronary interventions was later referred to cardiac transplantation due to terminal heart failure and the diagnosis of CS was revealed from the explanted heart. Additionally, one patient with pulmonary sarcoidosis and VTs underwent coronary bypass surgery and the diagnosis of cardiac sarcoid involvement was found in cardiac tissue samples taken during surgery.

Table 8. Associated disorders in 110 patients with CS (Kandolin et al., unpublished results).

Autoimmune disorders	
Hypothyroidism	11 (10%)
Type I diabetes mellitus	3 (3%)
Rheumatoid arthritis	1 (1%)
Inflammatory bowel disease	1 (1%)
Coeliac disease	1 (1%)
Multiple sclerosis	1 (1%)
Respiratory disease	
Asthma	10 (9%)
COPD	1 (1%)
Malignancy	
Thyroid cancer	2 (2%)
Breast cancer	2 (2%)
Colon cancer	1 (1%)
Lymphoma	1 (1%)
Thymoma	1 (1%)
Melanoma	1 (1%)
Myeloma	1 (1%)
Other	
Hypertension	26 (24%)
Dyslipidemia	17 (15%)
Type II diabetes mellitus	13 (12%)
Coronary artery disease (2 with medical treatment alone)	5 (5%)
Kidney insufficiency	1 (1%)

5.2.2 GCM (V)

The mean age of the 32 GCM patients was 52.5 ± 12.7 years, ranging from 35 to 69 years. The majority of them, 69% (22 patients) were female. Among the 32 patients with GCM, 19% (6 patients) had associated autoimmune disorders, including reactive arthritis, iritis, and thyreoiditis (n=1), coeliac disease (n=2), psoriasis (n=1), rheumatoid arthritis (n=1), and hypothyroidism (n=1). One patient with vitiligo had thymoma-associated orbital myositis, i.e., symptoms compatible with giant cell polymyositis. Furthermore, in accordance with genetic susceptibility, among the 32 patients, two women were sisters and they both had a medical history of coeliac disease. These two patients had a healthy brother and their first-degree relatives did not have evidence of autoimmune disease or cardiomyopathy.

5.3 Presenting manifestations (I)

The first clinical manifestations in CS and GCM are presented in **Table 9**. AV-block was the most frequent first manifestation in CS, presenting in 48 (44%) patients. Following AV-block in frequency, ventricular arrhythmias were the second most common manifestation, presenting in 33% patients. The third most common manifestation was symptomatic heart failure, presenting in 18%. The median LVEF at echocardiography in patients presenting with heart failure was 30% (range: 15-47%).

In patients with GCM, AV-block and heart failure were equally common first manifestations, in 31%, followed by ventricular arrhythmias in 25%. First manifestations in GCM are described in **Table 9**.

Table 9. Modes of presentation in 110 CS patients and 32 GCM patients.

First clinical Manifestation	All CS patients, N=110	Clinically isolated CS, n=71*	CS with extracardiac disease, n=39*	All GCM patients, n=32
2nd or 3rd degree AV-block	48 (44 %)	34 (48%)	14 (36%)	10 (31%)
VT or VF	36 (33%) VT 31 (28%) VF 5 (5%)	27 (38%)	9 (23%)	8 (25%) VT 7 (22%) VF 1 (3%)¶
Heart failure	20 (18%)	8 (11%)	12 (31%)	10 (31%)
Others	6 (6%)†	2 (3%)	4 (10%)	4 (13%)§

The data presents numbers of patients and (%).

CS= cardiac sarcoidosis, GCM= giant cell myocarditis, AV-block= atrioventricular block, VT= ventricular tachycardia, VF= ventricular fibrillation.

*For comparison between clinically isolated CS and CS with extracardiac disease, p=0.016.

¶Out-of-hospital cardiac arrest.

† Multiple ventricular premature beats (n=4), mitral regurgitation (n=1) and pericardial effusion (n=1).

§ Chest pain and ST-segment changes in ECG, mimicking acute myocardial infarction (n=3) and perimyocarditis (n=1).

Manifestations from outside the heart were observed in 39 (35%) CS patients. The lungs were the most commonly affected organ in 23 (21%) patients, followed by lymph nodes (n=8), skin (n=7), central nervous system (n=5), eyes (n=4), and liver (n=3).

The diagnostic delay, ie., the median time from first cardiac manifestation to histologic CS diagnosis, was 9.5 months (range 0.3-168) in the whole group of CS patients (n=110). In case where there was AV-block as the first presentation (n=48), the median diagnostic delay was 14 months, compared to six months in cases of heart failure or VT/VF as presenting manifestations (p=0.182).

5.3.1 Importance of CS and GCM in idiopathic AV-block (III)

A key discovery of this work was the significance of CS and GCM in the causal spectrum of AV-block in young and middle-aged adults. From the HUH pacemaker registry, we identified a total of 6420 patients who had undergone pacemaker implantation for 2nd or 3rd degree AV-block between 1999 and 2009. Patients <18 years were excluded because of the high proportion of congenital heart diseases and the low incidence of sarcoidosis in this age group. Respectively, patients aged >55 were excluded due to the high prevalence of conduction tissue degeneration. Consequently, there were 113 patients aged 18-55 years, and 61 of them had a known etiology of AV-block at the time of pacemaker implantation. The other etiologies are presented in **Figure 13** (Kandolin et al., unpublished results). The remaining 72 patients (with a median age of 47, 61% female) had unexplained AV-block at the baseline. After diagnostic studies, 18 of the 72 (25%, 95% CI 15-30%) patients with initially unexplained AV-block were diagnosed with either CS (n=14) or GCM (n=4). Additionally, four patients had echocardiographic and 18F-FDG-PET findings compatible with CS, but due to the lack of histologic verification, they were classified as having idiopathic AV-block and clinically probable CS. Considering all 2nd to 3rd degree AV-blocks in this age group, CS and GCM explained 14% (18 out of 133) and including the non-histologically proven CS cases, the percentage is 17% (22 out of 133).

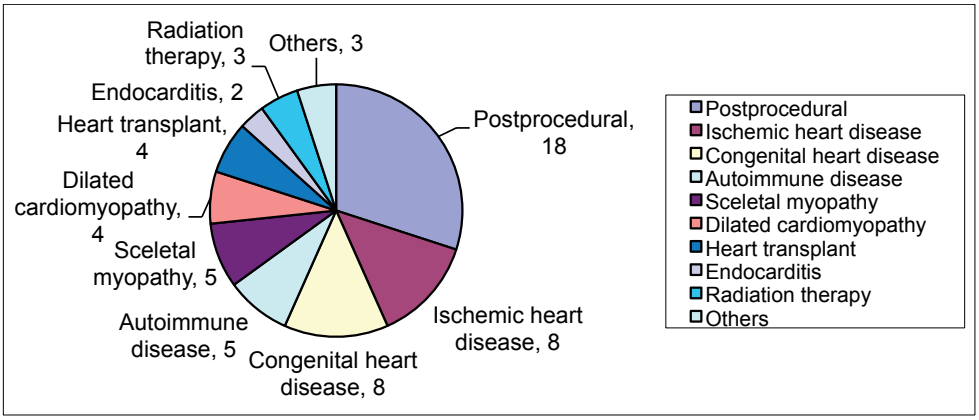


Figure 13. Etiologies for AV-block with a known cause at the time of pacemaker implantation (n=61) (Kandolin et al., unpublished results).

There were characteristic differences between the patients with CS or GCM compared to the patients in whom AV-block remained idiopathic after the diagnostic work-up. First, 89% of the patients with CS or GCM were female, compared to the 52% in patients with idiopathic AV-block ($p<0.05$). Other characteristic differences were lower LVEF at presentation (median 50% vs 61%, $p<0.05$), a higher percentage of distal AV-block (88% vs 54%, $p<0.01$) and a higher proportion of septal abnormalities at echocardiography (56% vs 28%, $p<0.01$) in CS and GCM patients compared to idiopathic AV-block patients. None of these characteristics differed between CS and GCM patients.

5.3.2 Clinically isolated CS (I)

In the total CS population of study I, 71 out of 110 (65%) had clinically isolated CS type (see 4.1.1). The remaining 39 patients (35%) had extracardiac manifestations. There were marked differences in patients with isolated and extracardiac disease types (**Table 10**). First, isolated CS was associated with female predominance. Second, the patients with isolated disease type had a higher frequency of LV dysfunction, LGE at CMR, and septal abnormalities indicating more widespread cardiac involvement. In line with this, the laboratory markers of granulomatous inflammation were higher in patients with extracardiac disease (see 5.6). Furthermore, isolated CS was related to worse survival free of cardiac death, transplantation, and aborted sudden death (see 5.8.2.1).

Table 10. Differences in patient characteristics in clinically isolated CS vs CS with extracardiac manifestations (I).

Characteristic	Clinically isolated CS, n=71	CS with known extracardiac disease, n=39	P*
Age, years (mean ± SD)	51±9	50±9	0.463
Gender, n of females	53 (75%)	18 (46%)	0.003
Echocardiographic findings			
LVEF <50%	49/71 (69%)	16/39 (41%)	0.004
LV dilatation	27/64 (42%)	16/38 (42%)	1.000
Septal thinning or thickening	48/70 (69%)	15/39 (38%)	0.002
CMR findings			
Late gadolinium enhancement	36/38 (95%)	13/21 (62%)	0.002
18F-FDG-PET findings			
Focal FDG uptake	34/46 (74%)	14/20 (70%)	0.743
Mediastinal lymph node FDG uptake	22/31 (71%)	8/11 (73%)	1.000
FDG uptake outside heart and mediastinum	12/30 (40%)	4/9 (44%)	1.000

CS= cardiac sarcoidosis, LVEF= left ventricle ejection fraction, LV= left ventricle, CMR=cardiac magnetic resonance imaging, 18-FDG-PET=18-fluorodexyglucose positron emission tomography.

5.4 Imaging and ECG findings (I)

5.4.1 Echocardiography

In the cohort of 110 CS patients, echocardiography was performed at least once in all patients, either at diagnosis or in case of post-mortem and post-transplantation diagnoses, before diagnosis. At baseline, 65 out of 110 (59%) patients had reduced LVEF (<50%) with median LVEF 45% ±14%. LV was dilated (LVEDD> 60 mm in men or > 55 mm in women) in 43 out of 102 (42%). Interventricular septal thinning or thickening was discovered in 63 out of 109 (58%). It is notable, that not all these findings were evident at the first performed echocardiography, but instead developed by the time of CS diagnosis. Other abnormalities detected were aneurysmatic wall motion, pericardial effusion (usually mild but requiring fenestration in one patient) and mild to moderate mitral insufficiency.

In the cohort of 32 patients with GCM, echocardiography was performed in all except one patient with sudden cardiac death as the first manifestation. Initially, LVEF was reduced (<50%) in 23 out of 32 (74%) with a median LVEF of 38 ±13%. LV dilatation was observed in nine (28%) patients. Septal thinning or thickening was discovered in 21 (68%) and LV aneurysms in three (10%) patients.

5.4.2 18F-FDG-PET

Of the 110 CS patients, 66 (60%) underwent 18F-FDG-PET as a part of diagnostic evaluation. Some 48 (73%) of them had focally increased myocardial FDG uptake and in 44 (67%), FDG uptake was superimposable upon a perfusion defect. Accumulation of FDG in mediastinal lymph nodes was detected in 30 of the 42 (71%) patients undergoing thoracic 18F-FDG-PET. In comparison, hilar or mediastinal lymphadenopathy was observed in 15 (14%) patients in native chest X-rays. In 18 patients, FDG-positive mediastinal lymph nodes offered a biopsy target in case of negative EMB and high suspicion of CS.

Of the 32 GCM patients, 12 (38%) underwent 18F-FDG-PET revealing focal FDG uptake in 10 (83%) and involving the septum in all. A concomitant perfusion defect was observed in nine and two patients had a perfusion defect without FDG accumulation.

5.4.3 LGE-CMR

Of the 110 CS patients, 59 (54%) underwent LGE-CMR as part of diagnostic process. Overall, LGE was observed in 49 (83%). Local wall thinning or thickening was detected in 29 out of 50 (58%). Other findings perceived were ventricular dysfunction, dilatation of ventricles, and aneurysmatic wall abnormalities. In the 32 GCM patients, CMR showed areas of LGE in each of the nine patients who underwent the study.

5.4.4 Electrocardiogram

An electrocardiogram was available in 107 out of 110 CS patients. At the time of diagnosis some abnormalities, including bundle branch blocks, any level of atrioventricular blocks, pathologic Q-waves or ST-T changes, voltage abnormalities, or frequent VPBs were observed in 92 (86%) patients. AV-block was the most common finding, present in 48 (45%). RBBB, observed in 40 (37%) patients, was almost twice as frequent as LBBB, found in 22 (21%) patients.

5.5 Biopsy confirmation (I,II,V)

In the national cohort of 110 CS patients, 92 patients had undergone one or more EMBs of the RV, or of both ventricles (I). Ultimately, the histologic confirmation of sarcoidosis was obtained from EMB in 55 (50%) patients. In 47 cases, biopsy verification was acquired from extracardiac biopsy sites, including PET-positive mediastinal lymph nodes (n=18), lungs (n=11), peripheral lymph nodes (n= 8), skin (n=5), liver (n=3), and central nervous system (n=2). In the remaining eight patients the histologic verification was obtained from explanted hearts at transplantation (n=6) or at autopsy (n=2).

The value of repeated EMBs and mediastinal lymph node biopsies in diagnosing CS was analyzed in the cohort of 55 patients with suspected CS undergoing diagnostic biopsies at HUH between 2000 and 2010 (II). In 33 patients (60%), the CS diagnosis was finally verified by biopsy, and in 22 (40%) the histologic confirmation remained unobtainable despite cardiac symptoms and imaging results compatible with CS. Considering the patients with eventual histological CS confirmation, the first EMB session revealed CS in 10 out of 31 patients, resulting in a sensitivity of 32%. A second and third EMB session contributed significantly to the diagnoses, as the cumulative yield of repeated EMBs was 55% (17 out of 31). In one patient who had not undergone diagnostic biopsies, the histologic diagnosis was made from the native heart removed at transplantation.

Due to the poor sensitivity of EMB, the diagnostic strategy at HUH was upgraded in 2006 to target 18F-FDG-PET-positive, i.e., “hot” mediastinal lymph nodes in case of high suspicion of CS despite negative EMBs. Twelve patients with “hot” mediastinal lymph nodes underwent sampling of these lymph nodes at mediastinoscopy and in 11 (92%), the samples identified sarcoidosis. Nevertheless, the 22 patients without final histological CS confirmation underwent a total of 33 EMBs and one mediastinal lymph node biopsy with negative results. If these patients were classified as having CS, in the same way as many recent studies have done, the sensitivity of multiple EMBs in this cohort would be 31.5% (17 out of 54). In the total population of biopsied patients, the sensitivity of EMB in histologically or clinically diagnosed CS was 19% (10 out of 53) in the first EMB, 22% (5 out of 23) in a second EMB and 50% (2 out of 4) in a third EMB. Over the study period, the mean number of right ventricular samples per biopsy session increased from 5.5 (range 4-7) during the early half (from 2000 to 2005) to 6.2 (range 3-10) over the latter half (years 2006-2010). The mean number of LV samples, acquired

only after 2006 but with increasing frequency, was 5.3 (range 2-9) per session. Open chest core needle biopsies were not performed in this cohort.

The diagnostic yield of EMB was better in GCM. Of the 32 GCM patients, 28 (88%) had undergone one or several EMBs (n=28) and surgical biopsies (n=3). The first cardiac biopsy session revealed GCM in 19 out of 28 cases, resulting in a diagnostic yield of 68%. The second cardiac biopsy gave diagnosis in five of seven patients, and a third cardiac biopsy in two of two patients. Therefore, the cumulative yield of repeated cardiac biopsies was 93% (26 out of 28). The three surgical myocardial biopsies were taken in association with surgical procedures (pericardial drainage, ventricle resection and ventricular assist device implantation), and the samples were positive in all. Lastly, of the 32 GCM patients, six of whom two had undergone EMBs with negative results, were diagnosed at autopsy (n=4) or from explanted hearts (n=2).

5.6 Laboratory findings (I, IV)

Hs-cTnT/I were repeatedly measured in a cohort of 62 CS patients with new-onset, treatment-naïve CS (IV). At enrollment, hs-cTnT was elevated (>13 ng/l) in 26 out of 50 (52%) and hs-cTnI (>0.04 ng/ml) in seven out of 12 (58%) of the newly diagnosed patients. Elevated hs-cTnT/I was associated with LV dysfunction (p=0.001), a tendency to higher NT-proBNP levels (p=0.085), and a tendency towards more heart failure (p=0.064) and less AV-block as a first manifestation (p=0.069). More specifically, in the new-onset CS patients, LVEF averaged 43± 14% in patients with elevated hs-cTnT/I compared to 53± 10% in patients with normal hs-cTnT/I.

The laboratory markers of granulomatous inflammation, including ACE, lysozyme and daily urinary calcium excretion in the 110 CS patients are presented in **Table 11**. The levels of these markers were higher in CS patients with extracardiac sarcoidosis compared to clinically isolated CS.

Table 11. Laboratory findings in CS patients (I).

Laboratory finding	All CS patients, n=110	Clinically isolated CS patients, n=71	CS with known extracardiac disease, n=39	P*
Elevated ACE	22/90 (24%)	9/59 (15%)	13/31 (42%)	0.005
Elevated LZM	40/74 (54%)	21/48 (44%)	19/26 (73%)	0.016
Elevated dU-Ca	22/45 (49%)	9/24 (38%)	13/21 (62%)	0.029

The data presents numbers of patients (%). CS= cardiac sarcoidosis, ACE= serum angiotensin-converting enzyme, LZM= serum lysozyme, dU-Ca= daily urinary calcium.
* p-values for comparison between clinically isolated and extracardiac disease.

5.7 Description of management (I,V)

5.7.1 Immunosuppressive treatment

All the 102 CS patients diagnosed clinically before death or cardiac transplantation received disease-modifying immunosuppressive therapy (I). There were slight differences between the hospitals involved in the study in implementing the treatment, but the therapy followed same main principles. Corticosteroids were started at diagnosis, with the initial daily prednisone-equivalent dose ranging from 30mg to 80mg. The starting dose was <60mg in 42 and ≥ 60mg in 60 patients. Corticosteroid dose was decreased slowly to a prednisone-equivalent daily dose of approximately 10mg within six months from starting treatment. In 48 patients, steroids were used continuously until the end of follow-up. In the remaining 54 patients, steroids were either discontinued or used intermittently due to fluctuating disease activity or steroid side effects. In patients with active disease following corticosteroid tapering, steroid-sparing immunosuppressants were introduced. Azathioprine was utilized in 50 patients, methotrexate in six patients, mycophenolate mofetil in three patients, cyclosporine in two patients and infliximab in one patient during the study period.

All the 26 patients diagnosed with GCM before death or transplantation received combined immunosuppression with 2-4 drugs (V). The treatment included steroids in 26 patients, azathioprine in 24 patients, cyclosporine in 20 patients, mycophenolate mofetil in three patients, muromonab in one patient, gammaglobulin in one patient, methotrexate in one patient and intravenous methylprednisolone in seven patients. The most common drug combination therapy was triple immunosuppression with corticosteroid, azathioprine, and cyclosporine utilized in 17 (65%) patients. Prednisone was started at approximately 60mg (or 0.75-1mg/kg) per day and tapered to a 10mg daily dose within six months. Prednisone was discontinued if the patient had been stabile for 6-12 months or in the case of intolerable side effects. The target dose of azathioprine was 1.5-2mg/kg/day. The target concentration of cyclosporine was at the lower therapeutic range for post-cardiac-transplantation (80-120 µg/L).

5.7.2 Device and medical therapy

During the study period, a permanent pacemaker was implanted in 87 out of 110 (79%) of CS patients (I). To prevent sudden cardiac death and to manage malignant VTs, an ICD was implanted in 59 (54%) patients. The indication for ICD was primary prevention (LVEF ≤ 35%) in nine (15%) patients, secondary prevention (history of sustained VT, including prior cardiac arrest) in 41 (69%), and other indication (treating clinician`s judgment) in nine (15%) patients (Kandolin et al., unpublished results). A biventricular pacemaker was implanted in 17 (15%) patients. The drug treatment in CS included beta blockers in 104 (95%) patients, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 89 (81%) patients, diuretics in 48 (44%), and amiodarone in 25 (23%) patients.

Of the 32 GCM patients, 11 (34%) received inotropic agents including levosimendan (n=6), dopamine, or dobutamine (n=5), norepinephrine (n=1), epinephrine (n=1), and milrinone (n=2) (Kandolin et al., unpublished results). Of the 26 GCM patients diagnosed before death or transplantation, 25 (96%) received beta blockers and 19 (73%) antiarrhythmic drugs (mainly amiodarone). A pacemaker was implanted in 21 (81%) and ICD in 18 (69%) patients.

5.8 Outcome (I,IV,V)

5.8.1 Observations during treatment and follow-up in CS

5.8.1.1 Clinical manifestations

The cohort of 110 CS patients was followed for a median of 79 months, i.e., 6.6 years (range 12-303 months) from first symptom onset. In addition to the 48 patients receiving a pacemaker for 2nd or 3rd degree AV-block as part of the early work-up, nine (8%) patients developed a new 2nd or 3rd degree conduction block necessitating pacemaker implantation later during the follow-up. Thus, a total of 52% of the whole CS cohort had significant atrioventricular conduction abnormalities during the course of the disease.

Furthermore, 15 (14%) patients experienced VF and 41 (37%) patients had sustained VT, either as first manifestation or later during the disease course. During the median follow-up of 53 months, i.e., 4.4 years (range 0-183 months) from ICD implantation until the close of the study period, 24 (41%) of the 59 patients with ICD received an appropriate ICD therapy (shock and/or anti-tachycardia pacing) (Kandolin et al., unpublished results).

Regarding systolic dysfunction, 74 (67%) of the patients either had systolic LV dysfunction (EF <50%) at diagnosis (n=65), or developed it later during the follow-up (n=9).

5.8.1.2 Treatment effect evaluation

The clinical response to corticosteroid therapy was evaluated from three aspects. First, initiation of corticosteroids, resulted in the recovery of atrioventricular conduction in 20% (7 of the 35 patients with accurate pacemaker follow-up data concerning the percentage of pacing) defined as <10% ventricular pacing on follow-up. Second, LV function at echocardiography was analyzed before and 12 months after the initiation of steroids. In the 102 patients receiving immunosuppressants, there was no difference in LVEF at diagnosis and after treatment (LVEF 44.9 ± 12% vs 45.4± 11%) (p=0.532). Nevertheless, in the subgroup of patients with severe LV impairment (EF <35%) at diagnosis, mild yet significant recovery of LV function was observed (LVEF before treatment 27.9± 4.1% vs LVEF 12 months after treatment 34.1± 8.3%) (p=0.005).

However, no amelioration of LVEF was perceived in the subgroups of normal LVEF (≥ 50%)(p=0.145) or moderately impaired LVEF (35-49%)(p=0.979).

During follow-up, 18F-FDG-PET was repeated in 22 (33%) patients to evaluate steroid response and disease activity (Kandolin et al., unpublished results). The 18F-FDG-PET images were analyzed mostly qualitatively and their diagnostic and prognostic value at follow-up was not considered beneficial for repeated studies with the protocol of the time. Therefore, the use of repeated 18F-FDG-PET studies decreased until the end of study period.

The early biochemical response to the initiation of corticosteroid therapy was assessed in the 62 patients with new-onset CS and multiple hs-cTnT/I measurements (IV). The initiation of treatment either decreased (n=8, 33%) or normalised (n=16, 67%) the hs-cTnT/I levels in four weeks in all the 24 patients with elevated pre-treatment concentrations who underwent repeated measurements after four (±1) weeks of steroid therapy. Hs-cTnT/I remained normal in all the 14 patients with normal pre-treatment concentrations (p=0.00003). **Figure 14** shows the hs-cTnT response to initiation of corticosteroid therapy in all 32 patients who underwent the one-month repeat measurement.

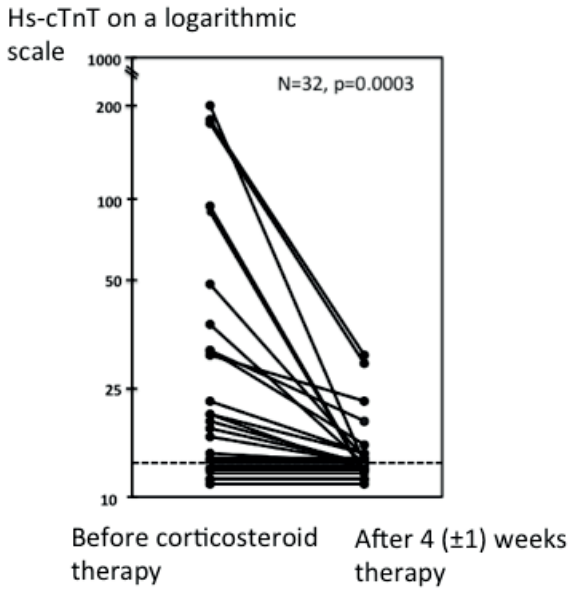


Figure 14. The early response of hs-cTnT to initiation of steroid therapy (IV).

The p-value is from a Wilcoxon signed rank test for paired comparisons. The dashed horizontal line signifies the upper limit of the normal reference range (≤13 ng/l).

During follow-up, three different profiles of serial hs-cTnT/I measurements could be distinguished (Kandolin et al., unpublished results). First, persistent hs-cTnT/I elevation was found in seven patients (11.3 %) of whom four had severe LV dysfunction (EF ≤35%). Second, 48 patients (77.4%) had either normal hs-cTnT/I in all measurements or initially elevated hs-cTnT/I that normalised and remained normal throughout the follow-up. The third group comprised of seven patients (11.3%) in whom hs-cTnT/I was initially normal, or normalised with treatment, but became elevated later during follow-up. In the group with late increases in hs-cTnT/I, the elevations were frequently preceded by a reduction in the intensity of immunosuppression and often responded to an increase in the dose or reinstitution of prednisone. **Figure 15** illustrates a case example of hs-cTnT evolution during corticosteroid treatment (Kandolin et al., unpublished results).

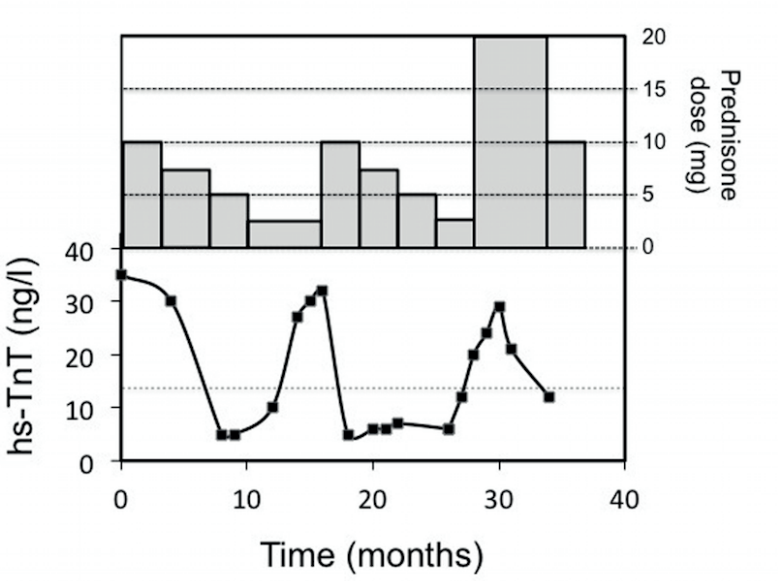


Figure 15. Serial hs-cTnT concentrations (lower panel) and daily prednisone dose (upper panel) during the three-year follow-up of a 43-year-old female with chronic CS. Hs-cTnT was elevated (>13 ng/l) at enrollment, and there were two additional late increases following reduction of the daily prednisone dose from 5mg to 2.5mg that responded each time to restoration of a higher dose (Kandolin et al., unpublished results).

Mild immunosuppressive treatment adverse effects, most commonly insomnia, edema, hypokalemia, impaired glucose tolerance due to corticosteroids and leukopenia, hepatic/pancreatic irritation, and nausea due to azathioprine, were commonly observed. Intolerable side effects (severe myopathy, psychosis, aseptic joint necrosis, and severe insomnia with diarrhea and hypokalemia) leading to permanent

discontinuation of corticosteroids were observed in four patients. No life-threatening adverse events were observed.

5.8.2 Serious outcome events and survival in CS

Figure 16 presents the serious outcome events in the cohort of 110 CS patients. A total of 10 (9%) patients suffered a cardiac death and six (5%) died of non-cardiac cause during the median follow-up of 79 months, i.e., 6.6 years (range 12-303 months) from symptom onset to death, transplantation or end of follow-up. Of the 10 cardiac deaths, nine were sudden deaths (in two cases diagnosis was not made until autopsy) and one due to terminal heart failure. The cardiac 1-, 5-, and 10-year Kaplan-Meier survival probabilities are presented in **Table 12** for the total population and for the 102 patients diagnosed clinically before death or transplantation and thus receiving immunosuppressive treatment.

During the follow-up 11 (10%) patients underwent a cardiac transplantation. Two of the transplanted patients died within one month of postoperative complications (multiple organ failure and intra-cerebral hemorrhage). Another two patients died of graft failure, without signs of sarcoidosis recurrence, three and five years post-transplantation. The remaining seven transplanted CS patients were alive without recurrence at the end of follow-up, median 60 months (range 22-249) from transplantation.

Consequently, at the end of median 6.6-year total follow-up from symptom onset, a total of 20 out of 110 (18%) patients had died and seven (6%) patients were alive with a transplanted heart. The cardiac survival free of transplantation is presented in **Table 12**. Additionally, two patients were awaiting transplantation, one with a ventricular assist device, at the close of follow-up. The main indication for transplantation was terminal heart failure in 11 listed and transplanted patients, and incontrollable ventricular arrhythmias in two, both of whom both also had severe heart failure (EF <35%). The time from symptom onset to transplantation ranged from five to 89 months, with a median of 59 months.

Furthermore, 11 (10%) other patients experienced an aborted sudden death as a first outcome event. Eight of them were resuscitated from VF and three received an ICD shock for VF. Thus, a total of 32 out of 110 (29%) patients suffered cardiac death, transplantation or aborted sudden death. **Table 12** also presents the survival rates without the composite event of cardiac death, transplantation and aborted sudden death.

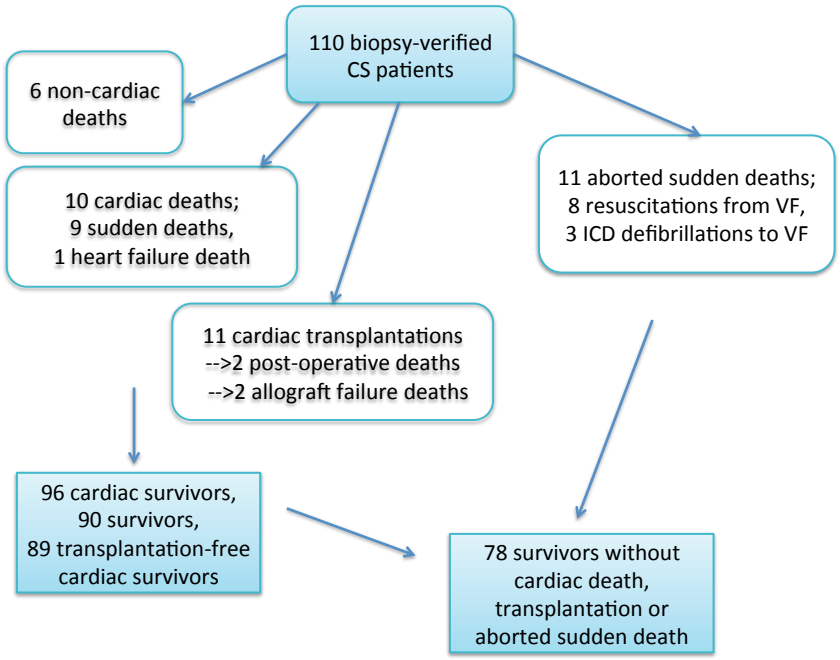


Figure 16. Serious outcome events in 110 CS patients (I).

Table 12. Survival free of major cardiac outcome events in all the 110 CS patients and in the 102 patients diagnosed prior to autopsy and transplantation (I).

	1-year survival, %	5-year survival, %	10-year survival, %
Cardiac survival			
n=110	99 (94-100)	94 (87-97)	89 (82-94)
n=102	100 (96-100)	97 (91-99)	93 (85-97)
Cardiac transplantation-free survival			
n=110	97 (92-99)	90 (82-95)	83 (75-89)
n=102	99 (94-100)	95 (88-98)	91 (83-95)
Patients with heart failure as first manifestation, n=20	90 (67-98)	75 (51-90)	53 (30-74)
Cardiac, transplantation- free and aborted sudden death-free survival			
n=110	89 (81-94)	78 (69-85)	70 (61-79)
n=102	89 (81-94)	82 (73-89)	77 (68-85)

CS= cardiac sarcoidosis.

5.8.2.1 Predictors of outcome

Heart failure as a primary manifestation was associated with worse transplant-free cardiac survival, and this applied across the total population (n=110) as well as in the clinically diagnosed (n=102) patients (log rank p=0.0001 and p=0.023, respectively). In the patients presenting with heart failure signs and symptoms, the 5-year Kaplan-Meier survival estimate was 75% (95% CI, 50.6- 90.4 %) compared to 90% (95% CI, 82.4-94.6%) in the total population (**Table 12**). On the other hand, AV-block as a first manifestation was associated with better outcome compared to other first manifestations (in the 102 patients, log rank p=0.037). Other predictors of cardiac survival free of transplantation in the total population of 110 patients were initial LVEF (p=0.006 by Cox regression and p=0.011 by log rank) and NYHA class at diagnosis (log rank p=0.023). On the contrary, the outcome was not related to age (Cox regression

p=0.469), sex (log rank p=0.163), or the type of CS (isolated vs extracardiac) (log rank p=0.132).

We also analyzed characteristics associated with survival free of transplantation and aborted sudden death. In the total CS cohort of 110 patients, LVEF was associated with survival free of this composite outcome event (by log rank p=0.046 and by Cox regression p=0.017). Instead, survival free of transplantation and aborted sudden death was independent of age, sex, and heart failure as the first manifestation. Furthermore, isolated CS type was related to worse survival free of this composite adverse event in the total population (n=110) and in the clinically diagnosed (n=102) patients (log rank p=0.005 and 0.015, respectively). This could be explained by the higher frequency of presentation with ventricular arrhythmias in isolated CS (see **Table 9**) and thus clustering of early aborted sudden deaths in this patient group.

Implementing prednisone treatment with a high (≥ 60mg/day) versus low (<60mg/day) initial prednisone dose did not effect survival free of cardiac death or transplantation (log rank p=0.561). Neither did the treatment delay from symptom onset to starting corticosteroids (<6 months vs ≥6 months) (log rank p=0.867). Furthermore, to evaluate the effect of corticosteroid in the subgroup of patients with AV-block as a first manifestation, we scrutinized the adverse cardiac events (cardiac death, aborted sudden death, sustained VT, new systolic LV dysfunction and transplantation) on follow-up in patients with early CS diagnosis and thus initiation of steroid treatment (<3 months from pacemaker implantation) compared to late diagnosis and initiation of steroid treatment (≥3 months from pacemaker implantation). During the follow-up of a median of 6.6 years, 50% (9 out of 18) in the early diagnosis group compared to 63% (17 out of 27) in the late diagnosis group had an adverse cardiac event, but the difference was not statistically significant (log rank p=0.821) (Kandolin et al., ESC Congress 2015 abstract 83429).

In the cohort of 62 newly diagnosed CS patients and serial hs-cTnT/I measurements at diagnosis and during follow-up (IV), there was a trend towards poorer prognosis in patients with elevated hs-cTnT/I at baseline (log rank p=0.068). More specifically, during the median follow-up of 17 months (1-48 months), a total of 16 out of 62 (26%) patients suffered a cardiac adverse event, including sudden cardiac death (n=2), aborted sudden cardiac death (n=5), symptomatic sustained VT (n=8), and new complete AV-block (n=1). In the group with elevated hs-cTnT/I, 11 out of 33 (33%) patients had an adverse event, compared to five out of 29 (17%) in the group with normal hs-cTnT/I at baseline.

5.8.3 Outcome in GCM (V)

The cohort of 32 GCM patients was followed for a median of 15 months (range 0.3-90.3 months) from symptom onset to death, transplantation or end of follow-up. During the follow-up, a total of 15 (47%) patients suffered cardiac death (n=5, 16%) or underwent cardiac transplantation (n=10, 31%). All the five fatalities were arrhythmic and four of

five patients who died were diagnosed post-mortem. The Kaplan-Meier estimates of transplant-free survival are presented in **Table 13.** In the subgroup of 26 patients diagnosed clinically before death or transplantation and thus receiving immunosuppressive therapy, one suffered cardiac death and eight underwent transplantation. Their transplant-free survival from diagnosis is also presented in **Table 13**.

Table 13. Survival free of major cardiac outcome events in all the 32 GCM patients and in the 26 patients diagnosed prior to autopsy and transplantation (V).

Cardiac* transplantation-free survival	1-year survival, %	5-year survival, %
n=32	69% (50-83%)	52% (34-70%)
n=26	77% (56-90%)	63% (42-81%)

* Same as transplantation-free survival. GCM= giant cell myocarditis.

All the eight patients with known pre-transplant diagnosis of GCM, were listed for transplantation within nine months from the diagnosis and the transplantations were accomplished within one year of diagnosis in seven of the eight patients. Of the 10 transplanted GCM patients, three died within six weeks of postoperative complications (large intracardiac thrombus (n=1), bleeding (n=1) and multiple organ failure (n=1)). The remaining seven transplanted GCM patients were alive at the close of follow-up. One patient had a recurrence of GCM in the graft, presenting as symptomatic heart failure 4.8 years post-transplantation, which ensued from discontinuing corticosteroid and was resolved with augmented immunosuppression.

The majority of GCM patients had ventricular arrhythmias. The aforementioned four patients suffered sudden arrhythmic death and were diagnosed only at autopsy. Of the two patients diagnosed at transplantation, one had sustained VTs. Of the 26 patients diagnosed before death and transplantation, 17 (65%) had sustained VTs, one was successfully resuscitated from VF, and one died of VF two weeks after diagnostic EMB. In total, 24 out of 32 (75%) of GCM patients experienced sudden cardiac death, aborted sudden cardiac death or sustained VT during the disease course. In the 18 GCM patients with ICD, eight (44%) received an appropriate ICD therapy (shock and/or anti-tachycardia pacing). In the subgroup of 17 transplant-free survivors, 10 (59%) had sustained VTs during follow-up and three received ICD shocks.

Considering systolic dysfunction in GCM, 28 out of 31 (90%) patients who had undergone echocardiography either had reduced LVEF (<50%) at diagnosis (n=23) or developed systolic dysfunction during the disease course (n=5). Symptomatic (NYHA II-IV) heart failure, with LVEF ranging from 20% to 40%, was observed in seven out of 26 patients with pre-transplant and pre-mortem GCM diagnosis. Six of them required

transplantation and one stabilized with medical therapy. Out of the four autopsy diagnosed patients, two had severe heart failure (EF ≤35%), one had normal echocardiography a year before sudden cardiac death, and one suffered sudden death without previous echocardiography. Both patients diagnosed at transplantation had severe systolic heart failure (EF <20%). Second or 3rd degree AV-block was present from the symptom onset in 11 (34%) patients (of whom in one the presenting symptom was classified as VT according to the principal symptom). None of the GCM patients developed a new AV-block during the study period.

Age, sex or presenting symptoms were not predictive of outcome in GCM. Initial LVEF was not statistically significantly associated with survival free of transplantation. Instead, at follow-up LVEF was higher in the transplant-free survivors (45±11%), compared to the patients who died or underwent transplantation (26±7%) (p=0.003).

6 DISCUSSION

6.1 Methodological considerations

6.1.1 Study population

This study is a retrospective analysis of CS and GCM patients in Finland. The CS patients were enlisted by contacting all the Finnish university and central hospitals. All the five university hospitals and six out of 17 central hospitals responded and screened the possible CS patients from their registries by using the given ICD-10 codes. The vast majority of diagnosed CS patients in Finland are treated and followed in university hospitals or have at least undergone diagnostic procedures in them during the diagnostic work-up. Thus the screening should have been comprehensive, yet some patients may have been missed due to deficiencies in hospital registries. Moreover, the ICD-10 classification was introduced in Finland in 1996, and therefore sporadic patients diagnosed between 1988 and 1996 who were lost during follow-up may have been dismissed.

There were differences in the prevalence of diagnosed CS between different health care districts (**Figure 10**). Although geographical divergence cannot be excluded, differences in diagnostic practices are the most likely cause. The diagnostic biopsy policy in patients with suspected inflammatory or other cardiomyopathy was particularly active in HUH, which is a national cardiac transplantation center, compared to other MIDFIN network hospitals. Of the 110 patients, 50% were from the HUH area, whereas the referral population of HUH represents roughly one-quarter of the total Finnish population. Outside HUH, patients were more often diagnosed clinically with CS and also treated without histopathological proof. Yet another limitation of the present study is that it was hospital-based and therefore sudden death in the community as a first manifestation of CS remained outside its scope. Finally, as a result of the active

diagnostic strategy, the proportion of autopsy and post-transplantation diagnoses (7%) was significantly lower than earlier studies (21-29%) (5,97) which results in differences in disease course.

The strict inclusion criteria requiring histologic verification of CS entails pros and cons. The inclusion policy applied results in diagnostic specificity as the patients included most definitely have CS. In contrast, a selection bias towards a more severe disease course might pursue, as the patients with a more severe disease are more rigorously examined and subsequently included in the study.

Four facts suggest that the true frequency of CS is probably higher than reported in this study. First, we did not include clinically diagnosed CS patients, the number of which might be double the number of histologically verified cases (272). The sensitivity of multiple EMBs in the HUH cohort was 31.5% (II) which is low but representative (2,21,86,91). Thus a considerable number of patients with suspect CS with negative EMBs or totally without histologic biopsies during the study period were left out of the scope of this study. Second, previous data show that symptomatic CS is just the tip of the iceberg (16,17,90,91,96,109) and that 20-30% of patients with extracardiac sarcoidosis have asymptomatic cardiac involvement (17,91,96). During the study period, systematic cardiac screening of patients with extracardiac sarcoidosis was not practiced in Finland. Therefore, in the epidemiologic sense a deficiency of this study is that it only involves the patients with symptomatic CS. Third, as indicated above, patients with outside-hospital sudden cardiac death as first a manifestation of CS could not be included. In the earlier autopsy series up to 35% of patients suffered cardiac death as a first manifestation of CS (16,17). However, with today's modern health care system the figure is likely to be lower. Fourth, as the response rate of the central hospitals was only 35%, some CS cases may have been missed. Anyhow, it is likely that the hospitals that failed to reply did not treat known CS cases and diagnosed cases from the corresponding areas were discovered from the university hospital records.

In respect to GCM, the data collection may have been incomplete as no specific ICD-10 code exits for GCM. Additionally, the fact that 50% (16 out of 32) of GCM patients were referred to HUH from other hospitals and 50% were from the HUH area, which covers approximately one-quarter of the Finnish population, also suggests that the true incidence of GCM in Finland is higher than presented in this study. Compared to previous studies, a significantly higher portion of GCM patients were diagnosed at biopsy before death or transplantation (81% vs 0-56%)(5,37). The higher percentage of lifetime diagnoses was probably a consequence of the active EMB policy at HUH and further reflects on differences in the clinical picture and prognosis compared to previous works.

6.1.2 Diagnostics

Diagnosing CS is challenging and can be pursued in three ways. Firstly, the golden standard and only absolute proof of CS is histologic confirmation of granulomatous

inflammation from the heart with other causes of granulomas excluded. However, EMB more often misses than hits the patchy granulomatous infiltrates with a sensitivity that is no better than 15-30% in previous studies (2,21,86,91) and 31.5% in our study. A second approach to CS diagnosis is by confirming histology from an extracardiac organ, e.g., lungs or lymph nodes together with symptoms and imaging findings compatible with CS. Third, a combination of typical imaging findings and symptoms together with clinically diagnosed extracardiac sarcoidois but without histologic confirmation has been considered to warrant diagnosis of CS (178). Anyhow, in this study a histologic verification either from the heart or an extracardiac organ was required, which is in accordance with the recently released CS guidelines (4). There are strong arguments pointing towards the fact that the confirmation of CS should be undisputed; the diagnosis triggers long-term immunosuppression with its potential side effects, commonly leads to ICD implantation, and portends a potentially life-threatening diagnosis in often otherwise healthy young individuals. Despite this, the initiation of treatment after repeated negative biopsies but strong clinical grounds could be justified on the basis that not treating might lead to permanent myocardial damage and potential fatality.

In terms of diagnosing CS from clinical perspective, there are three main scenarios of presentation. The first scenario comprises of patients presenting with cardiac conditions (mainly 2nd to 3rd degree AV-block, ventricular arrhythmias or unexplained heart failure usually together with conduction disturbances or VTs) without signs or symptoms of sarcoidosis from other organs. This used to be considered a rare presentation, but cumulating evidence from this study and others (108) shows that sarcoidosis often involves the heart without clinically apparent extracardiac disease. In this clinical setting, after ruling out coronary artery disease, a great deal of caution is needed to pursue the CS diagnosis, often with multiple EMBs and sometimes with targeting FDG-positive mediastinal lymph nodes for biopsy confirmation. It is notable that markers of granulomatous inflammation, including angiotensin-converting enzyme, lysozyme, and daily urinary calcium excretion were not of significant help in detecting CS. A second clinical scenario encompasses patients with biopsy-confirmed extracardiac sarcoidosis with symptoms of possibly cardiac origin. Given the positive histology in these cases, typical abnormalities in echocardiography, LGE-CMR, or cardiac 18F-FDG-PET confirm the CS diagnosis with sufficient certainty. A third scenario involves patients with biopsy-confirmed extracardiac sarcoidosis without cardiac symptoms. These patients should undergo medical history checks, a physical examination, and an ECG. Echocardiography may be considered but lacks sensitivity and is recommended mainly if one of the aforementioned screening tests is positive (88,197). Measuring hs-cTnT/I and NT-proBNP might be useful in guiding diagnostic decisions.

The diagnostic path in this study was different from the earlier reports in that the proportion of patients diagnosed at autopsy or from the transplanted heart (8 out of 110) was smaller (5,97). As mentioned previously, the explanation of the difference is

probably the active screening of young patients with AV-block and active EMB policy in unexplained cardiomyopathy with conduction abnormalities or arrhythmias at our institution. Furthermore, this was, to our knowledge, the first study to systematically utilize mediastinal lymph node biopsy in patients with negative EMB(s) and a high suspicion of CS.

CS biopsy findings were collected from hospital registries. Because histologic confirmation is the only absolute means of differentiating between GCM and CS (5), the GCM tissue samples were re-examined by cardiac pathologists for purposes of this study to ascertain accurate GCM diagnosis. In four cases the initial histologic diagnosis was converted from GCM to CS, which demonstrates the pitfalls in pathological diagnostics. On the other hand, the original histologic CS samples all contained granulomas and thus they were considered specific for CS.

6.1.3 Data

The data including patient characteristics, imaging and laboratory results, outcome events, etc., was principally collected from hospital charts and registries with a few exceptions. First, the ECGs were reanalyzed by the investigator when available. Second, the results of hs-cTnT/I measurements from other hospitals outside HUH were collected using questionnaires. The retrospective study design inherently causes deficiencies, such as lack of systematic diagnostic work-up and incompleteness of follow-up data. Anyhow, the potential deficiencies in clinical data were minimized by using complementary data collection systems (hospital discharge registries, the HUH pathology registry, the HUH pacemaker registry and the , Centre). Notably, we double-checked the most important outcome data (i.e. mortality) from the Population Register Centre. Furthermore, a single investigator (RK) scrutinized the patient data, thus enabling uniformity in data handling. Reporting the outcome, we focused on the hard end-points (death, transplantation, VF, VT, AV-block). Thus, we eluded the difficult-to-define end-points such as heart failure, which are problematic, especially in the case of retrospective study design.

All the echocardiographic data in this study was measured as a part of clinical work-up by various cardiology physicians, and a standardized echocardiography protocol was not applied. LVEF was defined by Simpson's method, M-mode and visual assessment. Echocardiographic analysis is known to be prone to inter-observer differences and incomplete reproducibility.

As to the diagnosis of isolated CS, screening for extracardiac sarcoidosis, such as ophthalmological and dermatological consultations, were not routinely conducted. Most importantly, chest (high-resolution) computed tomography was rarely performed. As a result, although these patients have isolated CS from the cardiologist's perspective, some of them probably have clinically silent extracardiac involvement.

Although the nature of this study was purely observational, the information gained over

the years was acknowledged by the treating physicians and has thus influenced the patient care. This particularly applies to the hs-cTnT/I study, where modulations of immunosuppressive treatment were conducted based on hs-cTnT/I alterations.

6.1.4 Analyses

The series of 110 CS patients is among the largest worldwide to date, and the series of 32 GCM patients is the largest involving pre-mortem diagnoses. Anyhow, from a statistical perspective the patient numbers and the numbers of outcome events are small, limiting the statistical power. All the studies were uncontrolled, thus making conclusions about treatment effect impossible. Furthermore, in respect to the sensitivity of diagnostic biopsies and imaging studies, the diagnoses of patients with negative results and thus the number of false-negative studies remained unknown. The outcome of these patients should be evaluated in further investigations.

6.2 Comparison with previous studies

6.2.1 Epidemiology of CS and GCM

Our study presents the first, yet limited population-based nationwide data on the prevalence and incidence of lifetime symptomatic CS. Until now, epidemiologic data on the prevalence of CS has been based on studies of lung sarcoidosis with cardiac involvement (31,77,79), and autopsy studies (16,17,49,109). We found that despite the marked increase in the detection rate over the 25-year period, CS still remained a very rare condition in Finland. The annual detection rate of symptomatic CS confirmed by biopsy was 0.6 per 100 000 adults and the prevalence 2.2 per 100 000 in 2012. Nevertheless, as discussed in the Methodological considerations section, the true prevalence of CS including clinically mild disease is likely to be significantly higher.

The most striking result to emerge from this study was the over 50-fold increase in the detection rate of CS over 26 years in Finland. This increase is likely to be explained by growing awareness of CS and advances in diagnostic methods. The fact that the largest increase in detection rate was dated over the years with the implementation of LGE-CMR and 18F-FDG-PET in clinical practice supports this theory. Despite this, a true increase in the incidence of CS, possibly due to an environmental or infectious factor, cannot be ruled out. Previous epidemiologic data on lung sarcoidosis showed similar incidence numbers in Finns compared to other white populations (74-76). Hence it can be assumed that our data is representable in other white populations, yet caution must be applied in generalizing these findings to other ethnic groups.

GCM is an even rarer myocardial disease, demonstrated by the fact that largest multicenter collaboration, collecting data from almost 40 centers worldwide between 1982 and 1997, could come up with no more than 73 GCM patients (5). The present GCM cohort of study V consisted of 32 patients, which is thus far the largest published single-center study (and our unpublished results add 17 new patients until end of 2014). Of the 32 GCM diagnoses, 29 were made over the latter 10 years of the study

period, with an annual detection rate of approximately 0.1 per 100 000. No prior epidemiologic lifetime data exists for comparison. Earlier autopsy studies reported incidences of 6.6-23.4 per 100 000 autopsies (25,102), but these figures might be overestimates since autopsies are usually performed in patients who have suffered unexpected death. On the other hand, it is possible that a clinically more benign form of GCM exists and its prevalence is higher than what is detected by EMBs and autopsies. The heterogeneity of disease manifestations observed in this study, as in the previous works (8,37), supports this theory.

6.2.2 Patient characteristics and disease manifestations

In general, our findings considering patient characteristics in CS are consistent with earlier observations. We found a female predominance of 65% and a mean age of 51± 9 years, in line with previous studies (5,113). Moreover, this study confirms the association of CS and GCM with autoimmune diseases. In line with previous studies, we found associated autoimmune disorders in 16% of CS patients and in 19% of GCM patients, which is higher than the 5-10% in the general population (273).

6.2.2.1 AV-block

One of the most significant observations from this study is the high prevalence (25%) of CS and GCM as causes of AV-blocks in adults aged <55 years. Thus, CS and GCM cause a far greater proportion of AV-blocks in young and middle-aged adults than previously thought. This result was later endorsed by a Canadian group that found CS in 34% (11 out of 32) patients with initially unexplained AV-block in patients aged 18-60 years (274). Furthermore, like us, Nery et al. found that the patients with AV-block due to CS had more severe cardiac events on follow-up compared to those in whom AV-block remained idiopathic (274). Consequently, it is important for the clinicians to consider the possibility of CS in all patients under 55-60 years with unexplained AV-block, even without arrhythmias or LV dysfunction at presentation. The diagnostic work-up of these patients should include LGE-CMR and/or 18F-FDG-PET.

6.2.2.2 Isolated CS

Even though CS without apparent extracardiac involvement has been reported before (5,16,22,87,109), our study was the first to discuss isolated CS as a clinical entity. In our nationwide cohort, two-thirds of patients had no apparent extracardiac involvement, which is consistent with a previous multicenter study (5). However, silent sarcoidosis outside the heart was not uncommonly detected in 18F-FDG-PET. Still, about one-third of patients undergoing whole-body 18F-FDG-PET scans did not have any extracardiac FDG accumulation. Since our study was published, isolated CS has attracted attention and variable frequencies of 5.5%-74% in CS have been presented (99,108,137,164,274). The highly variable percentages are likely due to different screening methods with

many centers routinely using whole-body 18F-FDG-PET, chest-computed tomography, ophthalmologic examination and close inspection of the skin in clinical work-up of CS and also actively screening patients with extracardiac sarcoidosis (164,274). In the largest multicenter CS study of 235 patients, chest-computed tomography was frequently performed and no less than 83.8% of patients had pulmonary sarcoidosis (129). It is possible that on a microscopical level, all sarcoidosis patients have lesions in extracardiac organs, but from a cardiologist's perspective, it is important to recognize the clinical entity of CS, as it makes diagnosis much more challenging. There are no sensitive biomarkers thus far to help in the detection of cardiac involvement in sarcoidosis, and further investigation is needed to assess the value of hs-cTnT/I in this respect.

In our cohort, more severe LV involvement and the female gender were associated with isolated CS. Recently, Tezuka et al. also found a correlation between lower LVEF and isolated CS (164). The female predominance might be explained by different disease manifestations between genders in myocarditis and cardiomyopathy (275). We found a worse event-free survival in isolated CS patients compared to systemic CS patients when aborted sudden death was included as an outcome event. This might be because of higher frequency of ventricular arrhythmias as the presenting manifestation in isolated CS. Although the reason for the VT proneness is unknown, similarly to our finding a recent large study of CS patients with ICD demonstrated 69% of patients with isolated CS receiving appropriate ICD therapies compared to 34% of patients with systemic CS (99). Finally, the overall clinical consequences of isolated CS remain to be elucidated in larger studies.

6.2.2.3 GCM and comparison with CS

In GCM, the mean age of 53 ± 13 years in our cohort was slightly higher than in previous studies, with a mean age of 42-48 years (5,9,11,63). This might have implications on prognosis, since young age is possibly linked to worse outcome in myocarditis (11,64,105). The GCM patients in our study were female in 69%, whereas the largest patient series showed no gender difference (5,11). It has been speculated that sex differences might play a role in myocarditis, with testosterone promoting more severe disease in men, but this theory has not been confirmed (275). In GCM patients, LVEF at diagnosis was reduced in 74% with a median of 38 ±13%, which is consistent with earlier reports (9,11,63). Moreover, we discovered some support for the genetic predisposition speculated in earlier studies (11,26,60), as two patients with as rare a disorder as GCM were siblings.

GCM is associated with fulminant onset and frequently fatal outcome (5,11), although a latent and prolonged clinical course has been described (5,8,37). Our study confirms the heterogenic nature of the disease. The presenting manifestations, including heart failure, ventricular arrhythmias, sudden death and conduction disturbances, were

comparable with previous studies, but their frequency was somewhat different. We found AV-block as the first manifestation in 31% of cases, which is higher than the 5-15% reported in prior multicenter studies (5,11). This difference might be explained by differences in diagnostic protocols between the earlier studies and ours, given that we applied an exceptionally proactive work-up in patients with AV-block. Thus, in contrast to earlier data (5), our finding implies that presentation with AV-block does not differentiate GCM from CS, although AV-block is more common in CS.

Previous data indicates that GCM and CS have many resemblances in etiology, pathophysiology and clinical picture, although they are distinctive clinicopathologic entities (5,7,26,27). Our findings on clinical manifestations and outcome support the previous concept that despite overlapping features, GCM and CS are separate diseases. More specifically, we observed a shorter median time from first cardiac manifestation to diagnosis of three months (range 0-16) in GCM, compared to 9.5 months (range 0.3-168) in CS, pointing towards a more rapid clinical course in GCM. At diagnosis, the patients with GCM had only slightly lower LVEF of 38 ±13%, in comparison with 45% ±14 in CS, but during the disease course, GCM patients more often developed systolic heart failure (90% vs 67%). Consistently, severe heart failure leading to transplantation was more common in GCM (31% vs 10%), and the arrhythmia burden was higher. Finally, the marked differences in survival rates discussed below are in line with previous works.

6.2.3 Disease course and the effect of treatment in CS

6.2.3.1 LV function

In line with other large studies (77,97,99,129), the median LVEF at the time of diagnosis was mildly reduced (45% ±14%) and patient series with both lower (115) and higher (6) initial EF exist. During the disease course, two-thirds of our patients had systolic dysfunction (defined as EF <50%) but data for comparison are lacking. Prior to clinical awareness and modern diagnostic methods, CS commonly manifested as symptomatic heart failure (19), but in our study as in other recent studies (93,146), overt heart failure was more rare. Similarly to other types of cardiomyopathies, ours and other CS studies (6,97,98,129) show that LVEF is an important prognostic factor in CS. In our study, the outcome was worst in patients with severe systolic dysfunction (EF <35%), but the difference was small between the patients with mildly reduced (EF 35-50%) and normal (>50%) EF. Furthermore, RV dysfunction has recently been reported to be not only common but also predisposing to ventricular arrhythmias in CS (98). However, since evaluating RV function by echocardiography is challenging and not routinely quantified, we did not analyze it.

6.2.3.2 AV-block

Overall, the high frequency of AV-conduction disturbances of 52% observed during the disease course of CS was comparable with previous reports (5,16,77,97). In small series

of up to 12 patients, the number of patients whose AV-conduction recovered in time or with corticosteroids was reported to be 33-75% (124,246,248) in comparison to 20% in our study. Besides small sample sizes, these differences might result from different definitions of AV-recovery, as in our study it was roughly estimated by <10% of ventricular pacing and in the studies focusing on assessing conduction, more specifically examined by Holter monitorings and ECGs.

6.2.3.3. Arrhythmias

CS presented as ventricular arrhythmias in 33% of patients, which is in line with previous and preceding studies (5,93,97,128,129). ICD was implanted in 54% of patients and the indication was secondary prevention in two-thirds of them, whereas in the recent studies from the U.S. over two-thirds of ICDs were implanted for primary prevention (98,128,129). After the study period, with accumulating information, the implantation protocol in Finland has changed towards a more active primary prevention strategy. At the median follow-up of 4.4 years from ICD implantation, 41% of the 59 Finnish CS patients with ICD received an appropriate ICD therapy, which corresponds to the therapy rate in recent studies (98,129). Furthermore, the increase in non-sustained VT frequency on follow-up should be regarded as a sign of potential disease activation.

6.2.3.4 Assessment of immunosuppressive treatment response

All the published clinical series suggest that corticosteroid therapy modifies the clinical course in CS (5,6,97,115,124,246,248,249). If treatment is initiated early on, systolic heart failure seems to develop rarely, whereas those with reduced LV function have a poor prognosis despite initiation of treatment (6,97). The corticosteroid treatment protocol in our study was not uniform but similar to older studies (6,97,244). Our study confirms that reduced LVEF is associated with worse prognosis despite immunosuppressive treatment. However, in contrast to earlier findings demonstrating benefit from corticosteroids in mild to moderate systolic heart failure (6,115) our data demonstrated a small yet significant improvement in LVEF in patients with severely reduced LVEF (<35%), but no change in normal or moderately depressed LVEF. The deficiency in our study as in others is that we do not know how LVEF would have resulted in the absence of corticosteroid treatment. Furthermore, the echocardiographic evaluation of LVEF is prone to divergence between operators, notably when LV is dilated. Similarly to earlier studies, no conclusions on survival benefit from immunosuppressants could be drawn, since all the patients diagnosed before death or transplantation received immunomodulatory treatment and respectively the ones without treatment were diagnosed at death or transplantation.

Concerning steroid-sparing agents, methotrexate is the most widely studied and used antimetabolite in sarcoidosis treatment (29,276). In contrast, in our cohort azathioprine was significantly more commonly used (50 vs six patients). There are differences in the side-effect profile between the two drugs. Azathioprine is associated with more infections, leading to discontinuation of the drug, leucopenia, nausea, and pancreatitis, whereas methotrexate involves more frequent hepatotoxic effects (276). An important aspect in choosing drug treatment is clinicians' user experience. For example, at HUH azathioprine has been used for a long time in treating cardiac transplant patients.

6.2.4 Serious outcome events and survival

6.2.4.1 In CS

Prior knowledge on CS prognosis is based on retrospective series and small prospective studies (5,16,22,97,109) mostly involving patients with extracardiac sarcoidosis screened for cardiac involvement (91,93,96). In the largest retrospective multicenter study by Yazaki et al. from Japan, the overall survival rate in CS was 60% at five years in all 95 patients and 75% in patients diagnosed before death (97). Similarly, the international collaboration study from Okura et al. reported transplant-free survival of 60% at five years in all 42 patients and 70% in patients diagnosed at EMB (5). In our cohort, the five-year transplant-free cardiac survival was 90% in all 110 patients and 95% in 102 patients diagnosed prior to death or transplantation. Although these figures cannot be directly compared since our numbers denote cardiac survival and the previous studies included non-cardiac deaths, the outcome seems more favorable in the present study. The possible reasons for better survival could be earlier diagnosis with shorter delay from symptom onset to diagnosis in our study (median 9.5 months, range 0.3-168 vs mean 29.7 ± 53.3 in the study of Okura et al.)(5). Alternatively, compared to the earlier studies, the immunosuppressive treatment has not changed markedly but there has been development in respect to arrhythmia treatment. In our study ICD was implanted in 54% vs 24% in the study by Okura et al. The high frequency of ventricular arrhythmias and particularly the high number of aborted sudden deaths in our study supports this theory. Lastly, differences in genetic backgrounds between the populations might have an impact on the outcome (49,75,277).

6.2.4.2 In GCM

Considering the outcome of GCM instead, our results suggest improved prognosis with combined immunosuppressive treatment. Previous data on the natural course of GCM demonstrated a grim prognosis, with a median transplant-free survival of three months from symptom onset but a significant survival benefit with a combination of immunosuppression therapy including cyclosporine (11). Comparably, an international multicenter study demonstrated a five-year transplant-free survival probability of 10% in all 73 patients and 22% transplant-free survival in 38 patients diagnosed before

death or transplantation and treated with at least corticosteroids (5). In our study the corresponding five-year survival probabilities of transplant-free survival were higher; 52% in all 32 patients and 63% in 26 patients receiving immunosuppressive therapy, respectively. Besides active and potent drug treatment with multiple immunosuppressants, the promptness in diagnostics and possibly demographic factors might explain the ameliorated survival rates in our study. Furthermore, similarly to CS, the increased frequency of ICDs might have improved the survival of GCM patients.

6.3 Clinical implications and future directions

The results of this work could have an impact on the diagnostics and management of CS and GCM patients in clinical practice. Most importantly, the finding that CS and GCM together cause 25% of initially unexplained AV-blocks in young to middle-aged patients (II) has been acknowledged by the authors of the AHA/ACCF/ESC EMB guidelines (234,278). Based on our results, they stated that EMB should probably be a class 2A recommendation (a reasonable procedure) in AV-block patients with a similar risk to CS/GCM (depending on age, ethnicity and exclusion of more common causes) (278). Second, in case of high suspicion of CS, imaging-guided biopsies of the heart or mediastinal lymph nodes, repeated if necessary, can be useful as they markedly increase the diagnostic yield (I,II). Sampling "hot" 18F-FDG-PET positive lymph nodes in mediastinoscopy has become clinical practice at HUH, since our findings demonstrated the high (close to 100%) sensitivity of this approach (279). Third, hs-cTnT/I have emerged as sensitive and easily repeatable markers that are routinely used at HUH as additional tools in assessing disease activity and treatment modifications in the follow-up of CS. Fourth, referring to our study (V), the 2015 ESC guidelines for the management of ventricular arrhythmias stated that the presence of malignant ventricular arrhythmias or heart block in GCM or CS might warrant earlier consideration of an ICD due to the known high risk of arrhythmic death or the need for transplantation (280).

An important aspect of this study lies in raising awareness and diagnostic activity towards CS and GCM. At best, increased knowledge enables earlier diagnosis and treatment prior to LV dysfunction, which in turn is likely to result in better outcome. Although rare diseases, CS and GCM should be kept in mind in cases with unexplained DCM, ventricular arrhythmias and AV-block, particularly in young to middle-aged adults.

Further research on CS and GCM is needed to elucidate the underlying mechanisms behind these enigmatic diseases and ultimately to improve the outcome. In enhancing diagnostic accuracy, new imaging methods, such as T1 mapping CMR (281) and novel PET isotopes (282) offer interesting targets. Moreover, more sensitive EMBs, using immunohistochemistry and molecularbiology methods (gene sequencing, RNA profiling etc.) are highly expected. Considering the treatment, assessing optimal drug regimen

and combinations should be studied. Possibly a similar treatment approach as in rheumatoid arthritis, involving rapid reductions in corticosteroid doses and a low threshold for monoclonal antibodies in treatment resistant cases, could be applied. However, the best regimen should be tested in randomized controlled trials. Large prospective multicenter registries are needed for the implication of the studies in both CS and GCM.

7 CONCLUSIONS

The present study was initiated by the clinical observation that the number of young people contracting CS, a disease with indeterminate etiology, diagnostics and outcome, was increasing at our institution. We found that:

1. The detection rate of CS increased over 50-fold over the 26-year study period from 1988 to 2014. The annual detection rate of biopsy-confirmed CS was 0.6 per 100 000 adults (>18 years) in the last two-year study period between 2012 and 2014. The prevalence of CS in 2012 was 2.2 per 100 000.

2. CS and GCM together explain 25% of 2nd to 3rd degree AV-blocks in adults aged 18-55 years in whom the etiology of AV-block is unknown at presentation.

3. Repeated, imaging-guided EMBs improve the diagnostic accuracy in CS. The sensitivity of first EMB was 31%, but second and third EMB sessions increased the sensitivity to 55%.

4. Sarcoidosis can manifest as a clinically isolated cardiac disorder without signs or symptoms from extracardiac organs. Two-thirds of CS patients had solely cardiac manifestations. Clinically isolated CS was characterized by more severe LV dysfunction and female predominance.

5. The outcome of CS with current diagnostics and treatment appears better than previously reported. In CS patients diagnosed before death or transplantation, the 10-year cardiac transplant-free survival was 91%.

6. Hs-cTnT/I are frequently elevated in new-onset CS. The concentrations rapidly and consistently decrease with corticosteroid treatment.

7. The prognosis of GCM with current diagnostics and treatment appears better compared to older studies. In GCM patients diagnosed before death or transplantation, the five-year cardiac transplant free survival was 63%.

8 ACKNOWLEDGEMENTS

This study was carried out at Helsinki University Hospital`s Heart and Lung Center and at the University of Helsinki in 2008-2015.

This scientific journey has taught me a great deal; firstly about scientific methods and ways of thinking, and secondly about myself and the ways of the world. Here I would like to express my acknowledgements to all the people with whom I have had the pleasure to connect with on this journey.

I owe my deepest gratitude to Professor Markku Kupari, whose ideas and visions have been the inspiration for these studies. His unique understanding of cardiology and his profound skills in statistics and scientific writing have been the cornerstones of this study.

I am indebted to Docent Jukka Lehtonen for his mentoring. His intelligence and multitasking skills never cease to amaze me. His unique sense of humor has been an irreplaceable source of joy during this project.

A growing group of researchers related to inflammatory heart diseases research has formed during the past few years. I wish to thank Piia Simonen for her energetic companionship and encourage Diana Velikanova, Kaj Ekström, Kaisa Nordenswan and Julia Raak to continue the good work together. Hopefully this thesis is just the beginning of a long and prosperous project. I would also like to thank all the co-authors and associate investigators in the hospitals across Finland who make up the MIDFIN research network. I owe gratitude to all the patients who participated in this study.

I am sincerely grateful to Docent Tuomas Rissanen and Docent Ville Kytö for their fast yet precise comments which helped me finalize this thesis. I deeply admire your scientific enthusiasm. I also wish to extend my gratitude to Nick Moon for revising the language.

I also wish to express my gratitude to my clinical colleagues in the cardiology department for their friendship, teaching and collaboration during my cardiology specialization.

I want to thank the cordial Dean Kimmo Kontula for letting me to use his research facilities at Biomedicum. Furhermore, I wish to thank the whole of Kontula's molecular medicine laboratory group, especially Annukka Lahtinen and Kati Donner for their support and letting me feel like I was a part their study group.

I would like to thank Docent Marit Graner and Docent Timo Hiltunen for practical help in statistics and scientific writing in the early important steps of my research career.

As peer support is priceless in life, I send my warmest thoughts to Laura Mäkinen, Sanja Sädevirta, Virpi Koskela-Niska, Milla Rosengård-Bärlund and Sanna Kaye, who are working on similar projects.

Heartfelt thanks go to my dear friends, especially Marika, Hanna, Taina, and Maarit for sharing the joys and sorrows of life. I extend my deepest gratitude to my sister Annamari; in knowing that she is around, I never feel alone in this world.

I would not be here without the strong foundations of independency, hard work and perseverance provided by my beloved parents Leena and Timo. Still today I can always count on their support, both mentally and in everyday matters which has recently involved a lot of taking care of Adrian. I also want to thank Susanne and Ismo, Kristiina and Christos, Heikki and Eila for being there.

Most importantly, my husband and best friend Johannes. Words cannot express the love, gratitude, and appreciation I feel towards you. You have supported me through good times and bad. Let the cardiac image you drew for me on the page 25 of this thesis symbolize our love, fragile yet strong as it is. My son Adrian, you were born in the middle of this study project and took a lot of attention away from it for which I am forever thankful. I love you.

This study was supported financially by the Finnish Foundation for Cardiovascular Research, Helsinki University EVO grants, the Finnish Cultural Foundation, and the Finnish-Norwegian Medical Foundation, and the congress presentations were supported by the Finnish Cardiac Society.

Helsinki, November 2015

Riina Kandolin

REFERENCES

1. Ayoub C, Pena E, Ohira H, Dick A, Leung E, Nery PB, et al. Advanced Imaging of Cardiac Sarcoidosis. *Curr Cardiol Rep*. 2015;17:17–12.

2. Uemura A, Morimoto S, Hiramitsu S, Kato Y, Ito T, Hishida H. Histologic diagnostic rate of cardiac sarcoidosis: evaluation of endomyocardial biopsies. *Am Heart J*. 1999;138:299–302.

3. Lagana SM, Parwani AV, Nichols LC. Cardiac sarcoidosis: a pathology-focused review. *Arch Pathol Lab Med*. 2010;134:1039–46.

4. Birnie DH, Sauer WH, Bogun F, Cooper JM, Culver DA, Duvernoy CS, et al. HRS expert consensus statement on the diagnosis and management of arrhythmias associated with cardiac sarcoidosis. *Heart Rhythm*. 2014;11:1305–23.

5. Okura Y, Dec GW, Hare JM, Kodama M, Berry GJ, Tazelaar HD, et al. A clinical and histopathologic comparison of cardiac sarcoidosis and idiopathic giant cell myocarditis. *J Am Coll Cardiol*. 2003;41:322–9.

6. Chiu C-Z, Nakatani S, Zhang G, Tachibana T, Ohmori F, Yamagishi M, et al. Prevention of left ventricular remodeling by long-term corticosteroid therapy in patients with cardiac sarcoidosis. *Am J Cardiol.* 2005;95:143–6.

7. Rosenstein ED, Zucker MJ, Kramer N. Giant cell myocarditis: most fatal of autoimmune diseases. *Semin Arthritis Rheum*. 2000;30:1–16.

8. Ren H, Poston RS, Hruban RH, Baumgartner WA, Baughman KL, Hutchins GM. Long survival with giant cell myocarditis. *Mod Pathol.* 1993;6:402–7.

9. Davidoff R, Palacios I, Southern J, Fallon JT, Newell J, Dec GW. Giant cell versus lymphocytic myocarditis. A comparison of their clinical features and long-term outcomes. *Circulation.* 1991;83:953–61.

10. Litovsky SH, Burke AP, Virmani R. Giant cell myocarditis: an entity distinct from sarcoidosis characterized by multiphasic myocyte destruction by cytotoxic T cells and histiocytic giant cells. *Mod Pathol.* 1996;9:1126–34.

11. Cooper LT, Berry GJ, Shabetai R. Idiopathic giant-cell myocarditis -natural history and treatment. Multicenter Giant Cell Myocarditis Study Group Investigators. *N Engl J Med*. 1997;336:1860–6.

12. Sharma OP. Sarcoidosis: a historical perspective. *Clin Dermatol.* 2007;25:232-241.

13. Doughan AR, Williams BR. Cardiac sarcoidosis. *Heart.* 2006;92:282–8.

14. Schaumann J. Sarcoidosis with prurigo. *Ann Dermatol Syphiligr.* 1953;80:457–76.

15. Longcope WT, Freiman DG. A study of sarcoidosis; based on a combined investigation of 160 cases including 30 autopsies from The Johns Hopkins Hospital and Massachusetts General Hospital. *Medicine.* 1952;31:1–132.

16. Matsui Y, Iwai K, Tachibana T, Fruie T, Shigematsu N, Izumi T, et al. Clinicopathological study of fatal myocardial sarcoidosis. *Ann N Y Acad Sci.* 1976;278:455–69.

17. Silverman KJ, Hutchins GM, Bulkley BH. Cardiac sarcoid: a clinicopathologic study of 84 unselected patients with systemic sarcoidosis. *Circulation.* 1978;58:1204–11.

18. Fleming HA, McMahon JN, McCarthy CF, Kelehan P. Sarcoid heart disease. *Br Heart J.* 1983;50:498.

19. Fleming HA. Sarcoid heart disease. *Br Med J.* 1986;292:1095–6.

20. Fleming HA, Bailey SM. Sarcoid heart disease. *J R Coll Physicians Lond.* 1981;15:245–53.

21. Ratner SJ, Fenoglio JJ, Ursell PC. Utility of endomyocardial biopsy in the diagnosis of cardiac sarcoidosis. *Chest.* 1986;90:528–33.

22. Fleming HA, Bailey SM. The prognosis of sarcoid heart disease in the United Kingdom. *Ann N Y Acad Sci.* 1986;465:543–50.

23. Cooper LT. Giant cell and granulomatous myocarditis. *Heart Fail Clin.* 2005;1:431–7.

24. Saltykow S. Uber diffuse Myokarditis. *Virchows Archiv fur Pathologische Anatomie.* 1905; 182:1–39.

25. Whitehead R. Isolated myocarditis. *Br Heart J.* 1965;27:220–30.

26. Blauwet LA, Cooper LT. Idiopathic giant cell myocarditis and cardiac sarcoidosis. *Heart Fail Rev.* 2013;18:733–46.

27. Davies MJ, Pomerance A, Teare RD. Idiopathic giant cell myocarditis -a distinctive clinico-pathological entity. *Br Heart J.* 1975;37:192–5.

28. Mantini N, Williams B, Stewart J, Rubinsztain L, Kacharava A. Cardiac sarcoid: a clinician's review on how to approach the patient with cardiac sarcoid. *Clin Cardiol.* 2012;35:410–5.

29. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet P-Y, Müller-Quernheim J. Sarcoidosis. *Lancet.* 2014;383:1155–67.

30. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H, Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med.* 2001;164:1885–9.

31. Johns CJ, Michele TM. The clinical management of sarcoidosis. A 50-year experience at the Johns Hopkins Hospital. *Medicine.* 1999;78:65–111.

32. Saidha S, Sotirchos ES, Eckstein C. Etiology of sarcoidosis: does infection play a role? *Yale J Biol Med.* 2012;85:133–41.

33. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med.* 2007;357:2153–65.

34. Dubrey S, Shah S, Hardman T, Sharma R. Sarcoidosis: the links between epidemiology and aetiology. *Postgrad Med J*. 2014;90:582–9.

35. Bargout R, Kelly RF. Sarcoid heart disease: clinical course and treatment. *Int J Cardiol.* 2004;97:173–82.

36. Tchernev G, Tana C, Schiavone C, Cardoso J-C, Ananiev J, Wollina U. Sarcoidosis vs. Sarcoid-like reactions: The Two Sides of the same Coin? *Wien Med Wochenschr.* 2014;164:247–59.

37. Davies RA, Veinot JP, Smith S, Struthers C, Hendry P, Masters R. Giant cell myocarditis: clinical presentation, bridge to transplantation with mechanical circulatory support, and long-term outcome. *J Heart Lung Transplant.* 2002;21:674–9.

38. Papadopoulos KI, Hörnblad Y, Liljebladh H, Hallengren B. High frequency of endocrine autoimmunity in patients with sarcoidosis. *Eur J Endocrinol.* 1996;134:331–6.

39. Fuse K, Kodama M, Ito M, Okura Y, Kato K, Hanawa H, et al. Polarity of helper T cell subsets represents disease nature and clinical course of experimental autoimmune myocarditis in rats. *Clin Exp Immunol.* 2003;134:403–8.

40. Hunninghake GW, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N Engl J Med.* 1981;305:429–34.

41. Moller DR. Cells and cytokines involved in the pathogenesis of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 1999;16:24–31.

42. Grunewald J, Eklund A. Role of CD4+ T cells in sarcoidosis. *Proc Am Thorac Soc.* 2007;4:461–4.

43. Grutters JC, van den Bosch JMM. Corticosteroid treatment in sarcoidosis. *Eur Respir J.* 2006;28:627–36.

44. Chen ES, Moller DR. Sarcoidosis -scientific progress and clinical challenges. *Nat Rev Rheumatol.* 2011;7:457–67.

45. Grunewald J, Eklund A, Hed J, Takahashi H, Wigzell H. Aspects on the alveolar accumulation of T cells in sarcoidosis. *Sarcoidosis.* 1992;9:142–4.

46. Zhou T, Zhang W, Sweiss NJ, Chen ES, Moller DR, Knox KS, et al. Peripheral blood gene expression as a novel genomic biomarker in complicated sarcoidosis. *PLoS ONE.* 2012;7:1-13.

47. Sverrild A, Backer V, Kyvik KO, Kaprio J, Milman N, Svendsen CB, et al. Heredity in sarcoidosis: a registry-based twin study. *Thorax.* 2008;63:894–6.

48. Rybicki BA, Iannuzzi MC, Frederick MM, Thompson BW, Rossman MD, Bresnitz EA, et al. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). *Am J Respir Crit Care Med*. 2001;164:2085–91.

49. Iwai K, Sekiguti M, Hosoda Y, DeRemee RA, Tazelaar HD, Sharma OP, et al. Racial difference in cardiac sarcoidosis incidence observed at autopsy. *Sarcoidosis.* 1994;11:26–31.

50. Sato H, Woodhead FA, Ahmad T, Grutters JC, Spagnolo P, van den Bosch JMM, et al. Sarcoidosis HLA class II genotyping distinguishes differences of clinical phenotype across ethnic groups. *Hum Mol Genet.* 2010;19:4100–11.

51. Spagnolo P, Grunewald J. Recent advances in the genetics of sarcoidosis. *J Med Genet.* 2013;50:290–7.

52. Ikezoe K, Handa T, Tanizawa K, Kubo T, Ito I, Sokai A, et al. A toll-like receptor 3 single nucleotide polymorphism in Japanese patients with sarcoidosis. *Tissue Antigens.* 2015;85:204–8.

53. Liu Y, Li H, Xiao T, Lu Q. Epigenetics in immune-mediated pulmonary diseases. *Clin Rev Allergy Immunol.* 2013;45:314–30.

54. Christophi GP, Caza T, Curtiss C, Gumber D, Massa PT, Landas SK. Gene expression profiles in granuloma tissue reveal novel diagnostic markers in sarcoidosis. *Exp Mol Pathol.* 2014;96:393–9.

55. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. *Eur Respir J.* 2007;30:508–16.

56. Eishi Y. Etiologic link between sarcoidosis and Propionibacterium acnes. *Respir Investig.* 2013;51:56–68.

57. Newman LS, Rose CS, Bresnitz EA, Rossman MD, Barnard J, Frederick M, et al. A case control etiologic study of sarcoidosis: environmental and occupational risk factors. *Am J Respir Crit Care Med.* 2004;170:1324–30.

58. Newman KL, Newman LS. Occupational causes of sarcoidosis. *Curr Opin Allergy Clin Immunol.* 2012;12:145–50.

59. Crowley LE, Herbert R, Moline JM, Wallenstein S, Shukla G, Schechter C, et al. "Sarcoid like" granulomatous pulmonary disease in World Trade Center disaster responders. *Am J Ind Med.* 2011;54:175–84.

60. Kittleson MM, Minhas KM, Irizarry RA, Ye SQ, Edness G, Breton E, et al. Gene

expression in giant cell myocarditis: Altered expression of immune response genes. *Int J Cardiol.* 2005;102:333–40.

61. Kodama M, Matsumoto Y, Fujiwara M, Masani F, Izumi T, Shibata A. A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. *Clin Immunol Immunopathol.* 1990;57:250–62.

62. Zhang S, Kodama M, Hanawa H, Izumi T, Shibata A, Masani F. Effects of cyclosporine, prednisolone and aspirin on rat autoimmune giant cell myocarditis. *J Am Coll Cardiol.* 1993;21:1254–60.

63. Elezkurtaj S, Lassner D, Schultheiss HP, Escher F. Vascular involvement in cardiac giant cell myocarditis: a new pathophysiological aspect. *Clin Res Cardiol.* 2013;103:161–3.

64. Caforio ALP, Calabrese F, Angelini A, Tona F, Vinci A, Bottaro S, et al. A prospective study of biopsy-proven myocarditis: prognostic relevance of clinical and aetiopathogenetic features at diagnosis. *Eur Heart J.* 2007;28:1326–33.

65. Asimaki A, Tandri H, Duffy ER, Winterfield JR, Mackey-Bojack S, Picken MM, et al. Altered desmosomal proteins in granulomatous myocarditis and potential pathogenic links to arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhythm Electrophysiol.* 2011;4:743–52.

66. Sabatine MS, Poh K-K, Mega JL, Shepard J-AO, Stone JR, Frosch MP. Case records of the Massachusetts General Hospital. Case 36-2007. A 31-year-old woman with rash, fever, and hypotension. *N Engl J Med*. 2007;357:2167–78.

67. Mitoff PR, Mesana TG, Mielniczuk LM, Grenon J, Veinot JP, Cooper LT, et al. Giant cell myocarditis in a patient with a spondyloarthropathy after a drug hypersensitivity reaction. *Can J Cardiol*. 2013;29:7–8.

68. Daniels PR, Berry GJ, Tazelaar HD, Cooper LT. Giant cell myocarditis as a manifestation of drug hypersensitivity. *Cardiovasc Pathol.* 2000;9:287–91.

69. Baig M, Hatrick R. Giant cell myocarditis with incessant ventricular arrhythmias treated successfully with methylprednisolone and rat antithymocyte globulin. *Cardiol Res Pract.* 2011; 2011:1-4.

70. Reich JM. A critical analysis of sarcoidosis incidence assessment. *Multidiscip Respir Med.* 2013;8:57.

71. Zipse MM, Sauer WH. Cardiac sarcoidosis. *Curr Cardiol Rep.* 2014;16:514–10.

72. Gribbin J, Hubbard RB, Le Jeune I, Smith CJP, West J, Tata LJ. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Thorax.* 2006;61:980–5.

73. Reich JM, Johnson RE. Incidence of clinically identified sarcoidosis in a northwest United States population. *Sarcoidosis Vasc Diffuse Lung Dis*. 1996;13:173–7.

74. Hillerdal G, Nöu E, Osterman K, Schmekel B. Sarcoidosis: epidemiology and prognosis. A 15-year European study. *Am Rev Respir Dis.* 1984;130:29–32.

75. Rybicki BA, Major M, Popovich J, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. *Am J Epidemiol.* 1997;145:234–41.

76. Pietinalho A, Hiraga Y, Hosoda Y, Löfroos AB, Yamaguchi M, Selroos O. The frequency of sarcoidosis in Finland and Hokkaido, Japan. A comparative epidemiological study. *Sarcoidosis.* 1995;12:61–7.

77. Smedema J-P, Snoep G, van Kroonenburgh MPG, van Geuns R-J, Dassen WRM, Gorgels AP, et al. Cardiac involvement in patients with pulmonary sarcoidosis assessed at two university medical centers in the Netherlands. *Chest.* 2005;128:30–5.

78. Sharma OP, Maheshwari A, Thaker K. Myocardial sarcoidosis. *Chest.* 1993;103:253–8.

79. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, Bois du R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis. 1999; 16: 149–73.

80. Joyce E, Ninaber MK, Katsanos S, Debonnaire P, Kamperidis V, Bax JJ, et al. Subclinical left ventricular dysfunction by echocardiographic speckle-tracking strain analysis relates to outcome in sarcoidosis. *Eur J Heart Fail.* 2015;17:51–62.

81. Darlington P, Gabrielsen A, Sörensson P, Cederlund K, Eklund A, Grunewald J. Cardiac involvement in Caucasian patients with pulmonary sarcoidosis. *Respir Res.* 2014;15:15.

82. Akashi H, Kato TS, Takayama H, Naka Y, Farr M, Mancini D, et al. Outcome of patients with cardiac sarcoidosis undergoing cardiac transplantation -single-center retrospective analysis. *J Cardiol.* 2012;60:407–10.

83. Zhang M, Tavora F, Huebner T, Heath J, Burke A. Allograft pathology in patients transplanted for idiopathic dilated cardiomyopathy. *Am J Surg Pathol.* 2012;36:389–95.

84. Roberts WC, Roberts CC, Ko JM, Filardo G, Capehart JE, Hall SA. Morphologic features of the recipient heart in patients having cardiac transplantation and analysis of the congruence or incongruence between the clinical and morphologic diagnoses. *Medicine.* 2014;93:211–35.

85. Luk A, Metawee M, Ahn E, Gustafsson F, Ross H, Butany J. Do clinical diagnoses correlate with pathological diagnoses in cardiac transplant patients? The importance of endomyocardial biopsy. *Can J Cardiol.* 2009;25:48–54.

86. Ardehali H, Howard DL, Hariri A, Qasim A, Hare JM, Baughman KL, et al. A positive endomyocardial biopsy result for sarcoid is associated with poor prognosis in patients with initially unexplained cardiomyopathy. *Am Heart J.* 2005;150:459–63.

87. Tavora F, Cresswell N, Li L, Ripple M, Solomon C, Burke A. Comparison of necropsy findings in patients with sarcoidosis dying suddenly from cardiac sarcoidosis versus dying suddenly from other causes. *Am J Cardiol.* 2009;104:571–7.

88. Hamzeh N, Steckman DA, Sauer WH, Judson MA. Pathophysiology and clinical management of cardiac sarcoidosis. *Nat Rev Cardiol.* 2015; 24:1–11.

89. Abeler V. Sarcoidosis of the cardiac conducting system. *Am Heart J.* 1979;97:701–7.

90. Nagai T, Kohsaka S, Okuda S, Anzai T, Asano K, Fukuda K. Incidence and prognostic significance of myocardial late gadolinium enhancement in patients with sarcoidosis without cardiac manifestation. *Chest.* 2014;146:1064–72.

91. Patel MR, Cawley PJ, Heitner JF, Klem I, Parker MA, Jaroudi WA, et al. Detection of myocardial damage in patients with sarcoidosis. *Circulation.* 2009;120:1969–77.

92. Patel AR, Klein MR, Chandra S, Spencer KT, Decara JM, Lang RM, et al. Myocardial damage in patients with sarcoidosis and preserved left ventricular systolic function: an observational study. *Eur J Heart Fail.* 2011;13:1231–7.

93. Mehta D, Lubitz SA, Frankel Z, Wisnivesky JP, Einstein AJ, Goldman M, et al. Cardiac involvement in patients with sarcoidosis: diagnostic and prognostic value of outpatient testing. *Chest.* 2008;133:1426–35.

94. Vignaux O, Dhote R, Duboc D, Blanche P, Devaux J-Y, Weber S, et al. Detection of myocardial involvement in patients with sarcoidosis applying T2-weighted, contrast-enhanced, and cine magnetic resonance imaging: initial results of a prospective study. *J Comput Assist Tomogr.* 2002;26:762–7.

95. Dhôte R, Vignaux O, Blanche P, Duboc D, Dusser D, Brezin A, et al. Value of MRI for the diagnosis of cardiac involvement in sarcoidosis. *Rev Med Interne.* 2003;24:151–7.

96. Greulich S, Deluigi CC, Gloekler S, Wahl A, Zürn C, Kramer U, et al. CMR imaging predicts death and other adverse events in suspected cardiac sarcoidosis. *JACC Cardiovasc Imaging.* 2013;6:501–11.

97. Yazaki Y, Isobe M, Hiroe M, Morimoto S-I, Hiramitsu S, Nakano T, et al. Prognostic determinants of long-term survival in Japanese patients with cardiac sarcoidosis treated with prednisone. *Am J Cardiol.* 2001;88:1006–10.

98. Schuller JL, Zipse M, Crawford T, Bogun F, Beshai J, Patel AR, et al. Implantable

cardioverter defibrillator therapy in patients with cardiac sarcoidosis. *J Cardiovasc Electrophysiol.* 2012;23:925–9.

99. Kron J, Sauer W, Mueller G, Schuller J, Bogun F, Sarsam S, et al. Outcomes of patients with definite and suspected isolated cardiac sarcoidosis treated with an implantable cardiac defibrillator. *J Interv Card Electrophysiol*. 2015;13:1–10.

100. Otsuka K, Terasaki F, Eishi Y, Shimomura H, Ogura Y, Horii T, et al. Cardiac sarcoidosis underlies idiopathic dilated cardiomyopathy: importance of mediastinal lymphadenopathy in differential diagnosis. *Circ J.* 2007;71:1937–41.

101. Chapelon-Abric C, de Zuttere D, Duhaut P, Veyssier P, Wechsler B, Huong DLT, et al. Cardiac Sarcoidosis. *Medicine.* 2004;83:315–34.

102. Okada R, Wakafuji S. Myocarditis in autopsy. *Heart Vessels Suppl.* 1985;1:23–9.

103. Kytö V, Saukko P, Lignitz E, Schwesinger G, Henn V, Saraste A, et al. Diagnosis and presentation of fatal myocarditis. *Hum Pathol.* 2005;36:1003–7.

104. Lassner D, Kühl U, Siegismund CS, Rohde M, Elezkurtaj S, Escher F, et al. Improved diagnosis of idiopathic giant cell myocarditis and cardiac sarcoidosis by myocardial gene expression profiling. *Eur Heart J.* 2014;35:2186–95.

105. Cooper LT. Giant Cell Myocarditis in Children. *Prog Pediatr Cardiol.* 2007;24:47–9.

106. Das BB, Recto M, Johnsrude C, Klein L, Orman K, Shoemaker L, et al. Cardiac transplantation for pediatric giant cell myocarditis. *J Heart Lung Transplant.* 2006;25:474–8.

107. Cooper LT, Hare JM, Tazelaar HD, Edwards WD, Starling RC, Deng MC, et al. Usefulness of Immunosuppression for Giant Cell Myocarditis. *Am J Cardiol.* 2008;102:1535–9.

108. Blankstein R, Osborne M, Naya M, Waller A, Kim CK, Murthy VL, et al. Cardiac Positron Emission Tomography Enhances Prognostic Assessments of Patients with Suspected Cardiac Sarcoidosis. *J Am Coll Cardiol.* 2013; 63:329-36.

109. Roberts WC, McAllister HA Jr, Ferrans VJ. Sarcoidosis of the heart: a clinicopathologic study of 35 necropsy patients (group I) and review of 78 previously described necropsy patients (group II). *Am J Med.* 1977;63:86-108.

110. Kim JS, Judson MA, Donnino R, Gold M, Cooper LT, Prystowski EN et al. Cardiac sarcoidosis. *Am Heart J.* 2009;157:9–21.

111. Dubrey SW, Falk RH. Diagnosis and management of cardiac sarcoidosis. *Prog Cardiovasc Dis.* 2010;52:336–46.

112. Morales AR, Levy S, Davis J, Fine G. Sarcoidosis and the heart. *Pathol Annu.* 1974;9:139–55.

113. Yazaki Y, Isobe M, Hiramitsu S, Morimoto S, Hiroe M, Omichi C, et al. Comparison of clinical features and prognosis of cardiac sarcoidosis and idiopathic dilated cardiomyopathy. *Am J Cardiol.* 1998;82:537–40.

114. Schuller JL, Olson MD, Zipse MM, Schneider PM, Aleong RG, Wienberger HD, et al. Electrocardiographic characteristics in patients with pulmonary sarcoidosis indicating cardiac involvement. *J Cardiovasc Electrophysiol.* 2011;22:1243–8.

115. Nagai T, Nagano N, Sugano Y, Asaumi Y, Aiba T, Kanzaki H, et al. Effect of Corticosteroid Therapy on Long-Term Clinical Outcome and Left Ventricular Function in Patients With Cardiac Sarcoidosis. *Circ J.* 2015;79:1593-600.

116. Sekhri V, Sanal S, Delorenzo LJ, Aronow WS, Maguire GP. Cardiac sarcoidosis: a comprehensive review. *Arch Med Sci.* 2011;7:546–54.

117. Yoshida Y, Morimoto S, Hiramitsu S, Tsuboi N, Hirayama H, Itoh T. Incidence of cardiac sarcoidosis in Japanese patients with high-degree atrioventricular block. *Am Heart J.* 1997;134:382–6.

118. Naruse Y, Sekiguchi Y, Nogami A, Okada H, Yamauchi Y, Machino T, et al. Systematic treatment approach to ventricular tachycardia in cardiac sarcoidosis. *Circ Arrhythm Electrophysiol.* 2014;7:407–13.

119. Uusimaa P, Ylitalo K, Anttonen O, Kerola T, Virtanen V, Pääkkö E, et al. Ventricular tachyarrhythmia as a primary presentation of sarcoidosis. *Europace.* 2008;10:760–6.

120. Furushima H, Chinushi M, Sugiura H, Kasai H, Washizuka T, Aizawa Y. Ventricular tachyarrhythmia associated with cardiac sarcoidosis: its mechanisms and outcome. *Clin Cardiol.* 2004;27:217–22.

121. Koplan BA, Soejima K, Baughman K, Epstein LM, Stevenson WG. Refractory ventricular tachycardia secondary to cardiac sarcoid: electrophysiologic characteristics, mapping, and ablation. *Heart Rhythm*. 2006;3:924–9.

122. Jefic D, Joel B, Good E, Morady F, Rosman H, Knight B, et al. Role of radiofrequency catheter ablation of ventricular tachycardia in cardiac sarcoidosis: report from a multicenter registry. *Heart Rhythm.* 2009;6:189–95.

123. Zipse MM, Sauer WH. Electrophysiologic manifestations of cardiac sarcoidosis. *Curr Opin Pulm Med.* 2013;19:485–92.

124. Banba K, Kusano KF, Nakamura K, Morita H, Ogawa A, Ohtsuka F, et al. Relationship between arrhythmogenesis and disease activity in cardiac sarcoidosis. *Heart Rhythm.* 2007;4:1292–9.

125. Freeman AM, Curran-Everett D, Weinberger HD, Fenster BE, Buckner JK, Gottschall EB, et al. Predictors of cardiac sarcoidosis using commonly available cardiac studies. *Am J Cardiol.* 2013;112:280–5.

126. Kumar S, Barbhaiya C, Nagashima K, Choi E-K, Epstein LM, John RM, et al.

Ventricular Tachycardia in Cardiac Sarcoidosis: Characterization of Ventricular Substrate and Outcomes of Catheter Ablation. *Circ Arrhythm Electrophysiol.* 2014;8:87–93.

127. Mc Ardle BA, Birnie DH, Klein R, de Kemp RA, Leung E, Renaud J, et al. Is there an association between clinical presentation and the location and extent of myocardial involvement of cardiac sarcoidosis as assessed by ^{18}F-fluorodoexyglucose positron emission tomography? *Circ Cardiovasc Imaging.* 2013;6:617–26.

128. Betensky BP, Tschabrunn CM, Zado ES, Goldberg LR, Marchlinski FE, Garcia FC, et al. Long-term follow-up of patients with cardiac sarcoidosis and implantable cardioverter-defibrillators. *Heart Rhythm.* 2012;9:884–91.

129. Kron J, Sauer W, Schuller J, Bogun F, Crawford T, Sarsam S, et al. Efficacy and safety of implantable cardiac defibrillators for treatment of ventricular arrhythmias in patients with cardiac sarcoidosis. *Europace.* 2013;15:347–54.

130. Forbes G, Usher A. Fatal Myocardial Sarcoidosis. *Br Med J.* 1962;2:771–1.

131. Nery PB, Mc Ardle BA, Redpath CJ, Leung E, Lemery R, Dekemp R, et al. Prevalence of cardiac sarcoidosis in patients presenting with monomorphic ventricular tachycardia. *Pacing Clin Electrophysiol*. 2014;37:364–74.

132. Winters SL, Cohen M, Greenberg S, Stein B, Curwin J, Pe E, et al. Sustained ventricular tachycardia associated with sarcoidosis: assessment of the underlying cardiac anatomy and the prospective utility of programmed ventricular stimulation, drug therapy and an implantable antitachycardia device. *J Am Coll Cardiol.* 1991;18:937–43.

133. Mehta D, Mori N, Goldbarg SH, Lubitz S, Wisnivesky JP, Teirstein A. Primary prevention of sudden cardiac death in silent cardiac sarcoidosis: role of programmed ventricular stimulation. *Circ Arrhythm Electrophysiol.* 2011;4:43–8.

134. Aizer A, Stern EH, Gomes JA, Teirstein AS, Eckart RE, Mehta D. Usefulness of programmed ventricular stimulation in predicting future arrhythmic events in patients with cardiac sarcoidosis. *Am J Cardiol.* 2005;96:276–82.

135. Vasaiwala SC, Finn C, Delpriore J, Leya F, Gagermeier J, Akar JG, et al. Prospective study of cardiac sarcoid mimicking arrhythmogenic right ventricular dysplasia. *J Cardiovasc Electrophysiol.* 2009;20:473–6.

136. Dechering DG, Kochhäuser S, Wasmer K, Zellerhoff S, Pott C, Köbe J, et al. Electrophysiological characteristics of ventricular tachyarrhythmias in cardiac sarcoidosis versus arrhythmogenic right ventricular cardiomyopathy. *Heart Rhythm.* 2013;10:158–64.

137. Nery PB, Keren A, Healey J, Leug E, Beanlands RS, Birnie DH. Isolated cardiac sarcoidosis: establishing the diagnosis with electroanatomic mapping-guided

endomyocardial biopsy. *Can J Cardiol.* 2013;29:1015.e1–3.

138. Philips B, Madhavan S, James CA, Riele te ASJM, Murray B, Tichnell C, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy and cardiac sarcoidosis: distinguishing features when the diagnosis is unclear. *Circ Arrhythm Electrophysiol.* 2014;7:230–6.

139. Cain MA, Metzl MD, Patel AR, Addetia K, Spencer KT, Sweiss NJ, et al. Cardiac sarcoidosis detected by late gadolinium enhancement and prevalence of atrial arrhythmias. *Am J Cardiol.* 2014;113:1556–60.

140. Willner JM, Viles-Gonzalez JF, Coffey JO, Morgenthau AS, Mehta D. Catheter ablation of atrial arrhythmias in cardiac sarcoidosis. *J Cardiovasc Electrophysiol.* 2014;25:958–63.

141. Viles-Gonzalez JF, Pastori L, Fischer A, Wisnivesky JP, Goldman MG, Mehta D. Supraventricular arrhythmias in patients with cardiac sarcoidosis prevalence, predictors, and clinical implications. *Chest*. 2013;143:1085–90.

142. Cordova FC, D'Alonzo G. Sarcoidosis-associated pulmonary hypertension. *Curr Opin Pulm Med.* 2013;19:531–7.

143. Desai MY, Fallert MA. Rapidly progressing congestive heart failure due to cardiac sarcoidosis involving papillary muscles: a case report and brief review of the literature. *Cardiol Rev.* 2003;11:163–8.

144. Miyazawa K, Yoshikawa T, Takamisawa I, Mahara K, Inoue K, Iguchi N, et al. Presence of ventricular aneurysm predicts poor clinical outcomes in patients with cardiac sarcoidosis. *Int J Cardiol.* 2014;177:720–2.

145. Schatka I, Bengel FM. Advanced imaging of cardiac sarcoidosis. *J Nucl Med.* 2014;55:99–106.

146. Schuller JL, Lowery CM, Zipse M, Aleong RG, Varosy PD, Weinberger HD, et al. Diagnostic utility of signal-averaged electrocardiography for detection of cardiac sarcoidosis. *Ann Noninvasive Electrocardiol.* 2011;16:70–6.

147. Hirota M, Yoshida M, Hoshino J, Kondo T, Isomura T. Sublocalization of Cardiac Involvement in Sarcoidosis and Surgical Exclusion in Patients With Congestive Heart Failure. *Ann Thorac Surg.* 2015;100:81–7.

148. Nakajima S, Hanazawa K, Kaitani K, Izumi C, Nakagawa Y, Ando K, et al. Cardiac resynchronization therapy is less effective in the patients with cardiac sarcoidosis: insights from Cubic study. *J Am Coll Cardiol.* 2013;61:E661.

149. Gasparovic H, Bueno R, Rybicki FJ, Baughmann KL, Capodilupo RC, Leacche M, et al. Sarcoidosis of the aortic root. *J Thorac Cardiovasc Surg*. 2004;128:761–2.

150. Cross B, Nicolarsen J, Bullock J, Sugeng L, Bardo D, Lang R. Cardiac sarcoidosis presenting as mitral regurgitation. *J Am Soc Echocardiogr.* 2007;20:906.e9–13.

151. Zoneraich S, Gupta MP, Mehta J, Zoneraich O, Wessely Z. Myocardial sarcoidosis presenting as acute mitral insufficiency. *Chest.* 1974;66:452–4.

152. Kinney E, Murthy R, Ascunce G, Donohoe R, Zelis R. Pericardial effusions in sarcoidosis. *Chest.* 1979;76:476–8.

153. Pesola G, Teirstein AS, Goldman M. Sarcoidois presenting with pericardial effusion. *Sarcoidosis.* 1987;4:42–4.

154. Zelcer AA, LeJemtel TH, Jones J, Stahl J. Pericardial tamponade in sarcoidosis. *Can J Cardiol.* 1987;3:12–3.

155. Darda S, Zughaib ME, Alexander PB, Machado CE, David SW, Saba S. Cardiac sarcoidosis presenting as constrictive pericarditis. *Tex Heart Inst J.* 2014;41:319–23.

156. Butany J, Bahl NE, Morales K, Thangaroopan M, Ross H, Rao V, et al. The intricacies of cardiac sarcoidosis: a case report involving the coronary arteries and a review of the literature. *Cardiovasc Pathol.* 2006;15:222–7.

157. Wait JL, Movahed A. Anginal chest pain in sarcoidosis. *Thorax.* 1989;44:391–5.

158. Kinney EL, Jackson GL, Reeves WC, Zelis R. Thallium-scan myocardial defects and echocardiographic abnormalities in patients with sarcoidosis without clinical cardiac dysfunction. An analysis of 44 patients. *Am J Med.* 1980;68:497–503.

159. Tellier P, Paycha F, Antony I, Nitenberg A, Valeyre D, Foult JM, et al. Reversibility by dipyridamole of thallium-201 myocardial scan defects in patients with sarcoidosis. *Am J Med.* 1988;85:189–93.

160. Lam CSP, Tolep KA, Metke MP, Glockner J, Cooper LT. Coronary sarcoidosis presenting as acute coronary syndrome. *Clin Cardiol.* 2009;32:e68–71.

161. Treaba DO, Benson MD, Assad LW, Dainauskas JR. Sarcoidosis and immunoglobulin lambda II light-chain amyloidosis diagnosed after orthotopic heart transplantation: a case report and review of the literature. *Mod Pathol.* 2005;18:451–5.

162. Iijima K, Chinushi M, Furushima H, Aizawa Y. Intramural inflammation as a cause of transient ST-segment elevation in a patient of cardiac sarcoidosis. *Europace.* 2012;14:300–2.

163. Gibbons WJ, Levy RD, Nava S, Malcolm I, Marin JM, Tardif C, et al. Subclinical cardiac dysfunction in sarcoidosis. *Chest.* 1991;100:44–50.

164. Tezuka D, Terashima M, Kato Y, Toriihara A, Hirasawa K, Sasaoka T, et al. Clinical Characteristics of Definite or Suspected Isolated Cardiac Sarcoidosis: Application of Cardiac Magnetic Resonance Imaging and (18)F-Fluoro-2-deoxyglucose Positron-Emission Tomography/Computerized Tomography. *J Card Fail.* 2015;21:313-22.

165. Isobe M, Tezuka D. Isolated cardiac sarcoidosis: Clinical characteristics, diagnosis and treatment. *Int J Cardiol.* 2015;182:132–40.

166. Maleszewski JJ, Orellana VM, Hodge DO, Kuhl U, Schultheiss H-P, Cooper LT. Long-Term Risk of Recurrence, Morbidity and Mortality in Giant Cell Myocarditis. *Am J Cardiol.* 2015;115:1733-8.

167. Graner M, Lommi J, Kupari M, Raisanen-Sokolowski A, Toivonen L. Multiple forms of sustained monomorphic ventricular tachycardia as common presentation in giant-cell myocarditis. *Heart.* 2007;93:119–21.

168. Larsen BT, Maleszewski JJ, Edwards WD, Cooper LT, Sobonya RE, Thompson VE, et al. Atrial giant cell myocarditis: a distinctive clinicopathologic entity. *Circulation.* 2013;127:39–47.

169. Burke JS, Medline NM, Katz A. Giant cell myocarditis and myositis. Associated with thymoma and myasthenia gravis. *Arch Pathol.* 1969;88:359–66.

170. Palmer HP, Michael IE. Giant-cell myocarditis with multiple organ involvement. *Arch Intern Med.* 1965;116:444–7.

171. Namba T, Brunner NG, Grob D. Idiopathic giant cell polymyositis. Report of a case and review of the syndrome. *Arch Neurol.* 1974;31:27–30.

172. Cooper LT, Okura Y. Idiopathic Giant Cell Myocarditis. *Curr Treat Options Cardiovasc Med.* 2001;3:463–7.

173. Perkel D, Czer LSC, Morrissey RP, Ruzza A, Rafiei M, Awad M, et al. Heart transplantation for end-stage heart failure due to cardiac sarcoidosis. *Transplant Proc.* 2013;45:2384–6.

174. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee. *Am J Respir Crit Care Med.* 1999;160:736–55.

175. Hiraga H, Yuwai K, Hiroe M, et al. Guideline for Diagnosis of Cardiac Sarcoidosis: Study Report on Diffuse Pulmonary Diseases From the Japanese Ministry of Health and Welfare. Tokyo: Japanese Ministry of Health and Welfare, 1993;23–24.

176. Soejima K, Yada H. The Work-Up and Management of Patients with Apparent or Subclinical Cardiac Sarcoidosis: With Emphasis on the Associated Heart Rhythm Abnormalities. *J Cardiovasc Electrophysiol.* 2009;20:578–83.

177. Judson MA, Costabel U, Drent M, Wells A, Maier L, Koth L, et al. The WASOG Sarcoidosis Organ Assessment Instrument: An update of a previous clinical tool. *Sarcoidosis Vasc Diffuse Lung Dis.* 2014;31:19–27.

178. Diagnostic standard and guidelines for sarcoidosis. *Jpn J Sarcoidosis*

Granulomatous Disord. 2007;27:89–102.

179. Kul S, Ozcelik HK, Uyarel H, Karakus G, Guvenc TS, Yalcınsoy M, et al. Diagnostic value of strain echocardiography, galectin-3, and tenascin-C levels for the identification of patients with pulmonary and cardiac sarcoidosis. *Lung.* 2014;192:533–42.

180. Matsumoto S, Hirayama Y, Saitoh H, Ino T, Miyauchi Y, Iwasaki Y-K, et al. Noninvasive diagnosis of cardiac sarcoidosis using microvolt T-wave alternans. *Int Heart J.* 2009;50:731–9.

181. Suzuki T, Kanda T, Kubota S, Imai S, Murata K. Holter monitoring as a noninvasive indicator of cardiac involvement in sarcoidosis. *Chest.* 1994;106:1021–4.

182. Burstow DJ, Tajik AJ, Bailey KR, DeRemee RA, Taliercio CP. Two-dimensional echocardiographic findings in systemic sarcoidosis. *Am J Cardiol.* 1989;63:478–82.

183. Uemura A, Morimoto S-I, Kato Y, Hiramitsu S, Ohtsuki M, Kato S, et al. Relationship between basal thinning of the interventricular septum and atrioventricular block in patients with cardiac sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 2005;22:63–5.

184. Nagano N, Nagai T, Sugano Y, Morita Y, Asaumi Y, Aiba T, et al. Association Between Basal Thinning of Interventricular Septum and Adverse Long-Term Clinical Outcomes in Patients With Cardiac Sarcoidosis. *Circ J.* 2015;79:1601–8.

185. Aydin Kaderli A, Gullulu S, Coskun F, Yilmaz D, Uzaslan E. Impaired left ventricular systolic and diastolic functions in patients with early grade pulmonary sarcoidosis. *Eur J Echocardiogr.* 2010;11:809–13.

186. Fahy GJ, Marwick T, McCreery CJ, Quigley PJ, Maurer BJ. Doppler echocardiographic detection of left ventricular diastolic dysfunction in patients with pulmonary sarcoidosis. *Chest.* 1996;109:62–6.

187. Sköld CM, Larsen FF, Rasmussen E, Pehrsson SK, Eklund AG. Determination of cardiac involvement in sarcoidosis by magnetic resonance imaging and Doppler echocardiography. *J Intern Med.* 2002;252:465–71.

188. Angomachalelis N, Hourzamanis A, Vamvalis C, Gavrielides A. Doppler echocardiographic evaluation of left ventricular diastolic function in patients with systemic sarcoidosis. *Postgrad Med J*. 1992;68:S52–6.

189. Focardi M, Picchi A, Nikiforakis N, Bargagli E, Fossi A, Maggiorelli C, et al. Assessment of cardiac involvement in sarcoidosis by echocardiography. *Rheumatol Int.* 2009;29:1051–5.

190. Youssef G, Beanlands RSB, Birnie DH, Nery PB. Cardiac sarcoidosis: applications of imaging in diagnosis and directing treatment. *Heart.* 2011;97:2078–87.

191. Tsuji T, Tanaka H, Matsumoto K, Miyoshi T, Hiraishi M, Kaneko A, et al. Capability of three-dimensional speckle tracking radial strain for identification of patients with cardiac sarcoidosis. *Int J Cardiovasc Imaging.* 2013;29:317–24.

192. Aggeli C, Felekos I, Tousoulis D, Gialafos E, Rapti A, Stefanadis C. Myocardial mechanics for the early detection of cardiac sarcoidosis. *Int J Cardiol.* 2013;168:4820–1.

193. Ohira H, Tsujino I, Ishimaru S, Oyama N, Takei T, Tsukamoto E, et al. Myocardial imaging with 18F-fluoro-2-deoxyglucose positron emission tomography and magnetic resonance imaging in sarcoidosis. *Eur J Nucl Med Mol Imaging.* 2008;35:933–41.

194. Smedema J-P, Truter R, de Klerk PA, Zaaiman L, White L, Doubell AF. Cardiac sarcoidosis evaluated with gadolinium-enhanced magnetic resonance and contrast-enhanced 64-slice computed tomography. *Int J Cardiol.* 2006;112:261–3.

195. Yoshida A, Ishibashi-Ueda H, Yamada N, Kanzaki H, Hasegawa T, Takahama H, et al. Direct comparison of the diagnostic capability of cardiac magnetic resonance and endomyocardial biopsy in patients with heart failure. *Eur J Heart Fail.* 2013;15:166–75.

196. Matoh F, Satoh H, Shiraki K, Odagiri K, Saitoh T, Urushida T, et al. The usefulness of delayed enhancement magnetic resonance imaging for diagnosis and evaluation of cardiac function in patients with cardiac sarcoidosis. *J Cardiol.* 2008;51:179–88.

197. Hamzeh NY, Wamboldt FS, Weinberger HD. Management of cardiac sarcoidosis in the United States: a Delphi study. *Chest.* 2012;141:154–62.

198. Yilmaz A, Ferreira V, Klingel K, Kandolf R, Neubauer S, Sechtem U. Role of cardiovascular magnetic resonance imaging (CMR) in the diagnosis of acute and chronic myocarditis. *Heart Fail Rev*. 2012;18:747–60.

199. Tadamura E, Yamamuro M, Kubo S, Kanao S, Saga T, Harada M, et al. Effectiveness of delayed enhanced MRI for identification of cardiac sarcoidosis: comparison with radionuclide imaging. *Am J Roentgenol*. 2005;185:110–5.

200. Serra JJ, Monte GU, Mello ES, Coral GP, Avila LFR, Parga JR, et al. Images in cardiovascular medicine. Cardiac sarcoidosis evaluated by delayed-enhanced magnetic resonance imaging. *Circulation*. 2003;107:e188–9.

201. Nadel J, Lancefield T, Voskoboinik A, Taylor AJ. Late gadolinium enhancement identified with cardiac magnetic resonance imaging in sarcoidosis patients is associated with long-term ventricular arrhythmia and sudden cardiac death. *Eur Heart J Cardiovasc Imaging*. 2015;16:634-41.

202. Crawford T, Mueller G, Sarsam S, Prasitdumrong H, Chaiyen N, Gu X, et al. Magnetic resonance imaging for identifying patients with cardiac sarcoidosis

and preserved or mildly reduced left ventricular function at risk of ventricular arrhythmias. *Circ Arrhythm Electrophysiol.* 2014;7:1109–15.

203. Ise T, Hasegawa T, Morita Y, Yamada N, Funada A, Takahama H, et al. Extensive late gadolinium enhancement on cardiovascular magnetic resonance predicts adverse outcomes and lack of improvement in LV function after steroid therapy in cardiac sarcoidosis. *Heart.* 2014;100:1165–72.

204. Shafee MA, Fukuda K, Wakayama Y, Nakano M, Kondo M, Hasebe Y, et al. Delayed enhancement on cardiac magnetic resonance imaging is a poor prognostic factor in patients with cardiac sarcoidosis. *J Cardiol.* 2012;60:448–53.

205. Watanabe E, Kimura F, Nakajima T, Hiroe M, Kasai Y, Nagata M, et al. Late gadolinium enhancement in cardiac sarcoidosis: characteristic magnetic resonance findings and relationship with left ventricular function. *J Thorac Imaging.* 2013;28:60–6.

206. Vignaux O, Dhote R, Duboc D, Blanche P, Dusser D, Weber S, et al. Clinical significance of myocardial magnetic resonance abnormalities in patients with sarcoidosis: a 1-year follow-up study. *Chest.* 2002;122:1895–901.

207. Yang Y, Safka K, Graham JJ, Roifman I, Zia MI, Wright GA, et al. Correlation of late gadolinium enhancement MRI and quantitative T2 measurement in cardiac sarcoidosis. *J Magn Reson Imaging.* 2014;39:609–16.

208. Youssef G, Leung E, Mylonas I, Nery P, Williams K, Wisenberg G, et al. The use of 18F-FDG PET in the diagnosis of cardiac sarcoidosis: a systematic review and metaanalysis including the Ontario experience. *J Nucl Med.* 2012;53:241–8.

209. Okumura W, Iwasaki T, Toyama T, Iso T, Arai M, Oriuchi N, et al. Usefulness of fasting 18F-FDG PET in identification of cardiac sarcoidosis. *J Nucl Med*. 2004;45:1989–98.

210. Mc Ardle BA, Leung E, Ohira H, Cocker MS, deKemp RA, DaSilva J, et al. The role of F18-fluorodeoxyglucose positron emission tomography in guiding diagnosis and management in patients with known or suspected cardiac sarcoidosis. *J Nucl Cardiol.* 2013;20:297–306.

211. Yamagishi H, Shirai N, Takagi M, Yoshiyama M, Akioka K, Takeuchi K, et al. Identification of cardiac sarcoidosis with (13)N-NH(3)/(18)F-FDG PET. *J Nucl Med.* 2003;44:1030–6.

212. Ishimaru S, Tsujino I, Takei T, Tsukamoto E, Sakaue S, Kamigaki M, et al. Focal uptake on 18F-fluoro-2-deoxyglucose positron emission tomography images indicates cardiac involvement of sarcoidosis. *Eur Heart J.* 2005;26:1538–43.

213. Ahmadian A, Brogan A, Berman J, Sverdlov AL, Mercier G, Mazzini M, et al. Quantitative interpretation of FDG PET/CT with myocardial perfusion imaging increases diagnostic information in the evaluation of cardiac sarcoidosis. *J Nucl*

Cardiol. 2014;21:925–39.

214. Osborne MT, Hulten EA, Singh A, Waller AH, Bittencourt MS, Stewart GC, et al. Reduction in ^{18}F-fluorodeoxyglucose uptake on serial cardiac positron emission tomography is associated with improved left ventricular ejection fraction in patients with cardiac sarcoidosis. *J Nucl Cardiol*. 2014;21:166–74.

215. Takaya Y, Kusano KF, Nakamura K, Kaji M, Shinya T, Kanazawa S, et al. Reduction of myocardial inflammation with steroid is not necessarily associated with improvement in left ventricular function in patients with cardiac sarcoidosis: predictors of functional improvement. *Int J Cardiol.* 2014;176:522–5.

216. Sobic-Saranovic D, Artiko V, Obradovic V. FDG PET imaging in sarcoidosis. *Semin Nucl Med.* 2013;43:404–11.

217. Langah R, Spicer K, Gebregziabher M, Gordon L. Effectiveness of prolonged fasting 18f-FDG PET-CT in the detection of cardiac sarcoidosis. *J Nucl Cardiol.* 2009;16:801–10.

218. Bulkley BH, Rouleau JR, Whitaker JQ, Strauss HW, Pitt B. The use of 201thallium for myocardial perfusion imaging in sarcoid heart disease. *Chest.* 1977;72:27–32.

219. Hirose Y, Ishida Y, Hayashida K, Maeno M, Takamiya M, Ohmori F, et al. Myocardial involvement in patients with sarcoidosis. An analysis of 75 patients. *Clin Nucl Med.* 1994;19:522–6.

220. Le Guludec D, Menad F, Faraggi M, Weinmann P, Battesti JP, Valeyre D. Myocardial sarcoidosis. Clinical value of technetium-99m sestamibi tomoscintigraphy. *Chest.* 1994;106:1675–82.

221. Kiuchi S, Teraoka K, Koizumi K, Takazawa K, Yamashina A. Usefulness of late gadolinium enhancement combined with MRI and 67-Ga scintigraphy in the diagnosis of cardiac sarcoidosis and disease activity evaluation. *Int J Cardiovasc Imaging.* 2007;23:237–41.

222. Tawarahara K, Kurata C, Okayama K, Kobayashi A, Yamazaki N. Thallium-201 and gallium 67 single photon emission computed tomographic imaging in cardiac sarcoidosis. *Am Heart J.* 1992;124:1383–4.

223. Okayama K, Kurata C, Tawarahara K, Wakabayashi Y, Chida K, Sato A. Diagnostic and prognostic value of myocardial scintigraphy with thallium-201 and gallium-67 in cardiac sarcoidosis. *Chest.* 1995;107:330–4.

224. Blauwet LA, Cooper LT. Idiopathic giant cell myocarditis and cardiac sarcoidosis. *Heart Fail Rev.* 2012;18:733–46.

225. Berthelot-Richer M, O'Connor K, Bernier M, Trahan S, Couture C, Dubois M, et al. When should we consider the diagnosis of giant cell myocarditis? Revisiting

"classic" echocardiographic and clinical features of this rare pathology. *Exp Clin Transplant.* 2014;12:565–8.

226. Hori T, Fujiwara H, Tanaka M, Matsuda M, Tsuyuguchi N, Suoh M. Idiopathic giant cell myocarditis accompanied by asymmetric septal hypertrophy. *Jpn Circ J.* 1987;51:153–6.

227. Vaideeswar P, Cooper LT. Giant cell myocarditis: clinical and pathological features in an Indian population. *Cardiovasc Pathol.* 2013;22:70–4.

228. Azarine A, Guillemain R, Bruneval P. Different focal delayed gadolinium-enhancement patterns using cardiac magnetic resonance in a case of diffuse giant cell myocarditis. *Eur Heart J.* 2009;30:1485.

229. Sujino Y, Kimura F, Tanno J, Nakano S, Yamaguchi E, Shimizu M, et al. Cardiac magnetic resonance imaging in giant cell myocarditis: intriguing associations with clinical and pathological features. *Circulation*. 2014;129:e467–9.

230. Pöyhönen P, Holmström M, Kivistö S, Hänninen H. Late gadolinium enhancement on CMR and sustained ventricular tachycardia predict severe cardiac inflammation. *Acta Cardiol*. 2014;69:637–47.

231. Caforio ALP, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*. 2013; 34:2636–48.

232. Childs H, Friedrich MG. Cardiovascular magnetic resonance imaging in myocarditis. *Prog Cardiovasc Dis.* 2011;54:266–75.

233. Mahrholdt H, Goedecke C, Wagner A, Meinhardt G, Athanasiadis A, Vogelsberg H, et al. Cardiovascular magnetic resonance assessment of human myocarditis: a comparison to histology and molecular pathology. *Circulation.* 2004;109:1250–8.

234. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Endorsed by the Heart Failure Society of America and the Heart Failure Association of the European Society of Cardiology. *J Am Coll Cardiol.* 2007;50:1914–31.

235. Liang JJ, Hebl VB, DeSimone CV, Madhavan M, Nanda S, Kapa S, et al. Electrogram Guidance: A Method to Increase the Precision and Diagnostic Yield of Endomyocardial Biopsy for Suspected Cardiac Sarcoidosis and Myocarditis. *JACC Heart Fail.* 2014;2:466–73.

236. Shields RC, Tazelaar HD, Berry GJ, Cooper LT. The role of right ventricular endomyocardial biopsy for idiopathic giant cell myocarditis. *J Card Fail.*

2002;8:74–8.

237. Yilmaz A, Kindermann I, Kindermann M, Mahfoud F, Ukena C, Athanasiadis A, et al. Comparative evaluation of left and right ventricular endomyocardial biopsy: differences in complication rate and diagnostic performance. *Circulation.* 2010;122:900–9.

238. Chimenti C, Frustaci A. Contribution and risks of left ventricular endomyocardial biopsy in patients with cardiomyopathies: a retrospective study over a 28-year period. *Circulation.* 2013;128:1531–41.

239. Konecny T, Noseworthy PA, Kapa S, Cooper LT, Mulpuru SK, Sandhu GS, et al. Endomyocardial biopsy-integrating electrode at the bioptome tip. *Ther Adv Cardiovasc Dis.* 2015;9:66-9.

240. Yasutake H, Seino Y, Kashiwagi M, Honma H, Matsuzaki T, Takano T. Detection of cardiac sarcoidosis using cardiac markers and myocardial integrated backscatter. *Int J Cardiol.* 2005;102:259–68.

241. Baba Y, Kubo T, Kitaoka H, Okawa M, Yamanaka S, Kawada Y, et al. Usefulness of high-sensitive cardiac troponin T for evaluating the activity of cardiac sarcoidosis. *Int Heart J.* 2012;53:287–92.

242. Tanada Y, Sato Y, Sawa T, Fujiwara H, Takatsu Y. Serial measurement of high-sensitivity cardiac troponin I and N-terminal proB-type natriuretic peptide in a patient presenting with cardiac sarcoidosis. *Intern Med.* 2012;51:3379–81.

243. De Boer RA, van der Velde AR, Mueller C, van Veldhuisen DJ, Anker SD, Peacock WF, et al. Galectin-3: a modifiable risk factor in heart failure. *Cardiovasc Drugs Ther.* 2014;28:237–46.

244. Sadek MM, Yung D, Birnie DH, Beanlands RS, Nery PB. Corticosteroid therapy for cardiac sarcoidosis: a systematic review. *Can J Cardiol.* 2013;29:1034–41.

245. Adcock IM. Molecular mechanisms of glucocorticosteroid actions. *Pulm Pharmacol Ther.* 2000;13:115–26.

246. Kato Y, Morimoto S-I, Uemura A, Hiramitsu S, Ito T, Hishida H. Efficacy of corticosteroids in sarcoidosis presenting with atrioventricular block. *Sarcoidosis Vasc Diffuse Lung Dis.* 2003;20:133–7.

247. Kudoh H, Fujiwara S, Shiotani H, Kawai H, Hirata K. Myocardial washout of 99mTc-tetrofosmin and response to steroid therapy in patients with cardiac sarcoidosis. *Ann Nucl Med.* 2010;24:379–85.

248. Yodogawa K, Seino Y, Shiomura R, Takahashi K, Tsuboi I, Uetake S, et al. Recovery of atrioventricular block following steroid therapy in patients with cardiac sarcoidosis. *J Cardiol.* 2013;62:320–5.

249. Yodogawa K, Seino Y, Ohara T, Takayama H, Katoh T, Mizuno K. Effect of corticosteroid therapy on ventricular arrhythmias in patients with cardiac

sarcoidosis. *Ann Noninvasive Electrocardiol.* 2011;16:140–7.

250. Nagai S, Yokomatsu T, Tanizawa K, Ikezoe K, Handa T, Ito Y, et al. Treatment with methotrexate and low-dose corticosteroids in sarcoidosis patients with cardiac lesions. *Intern Med.* 2014;53:427–33.

251. Barnabe C, McMeekin J, Howarth A, Martin L. Successful treatment of cardiac sarcoidosis with infliximab. *J Rheumatol.* 2008;35:1686–7.

252. Uthman I, Touma Z, Khoury M. Cardiac sarcoidosis responding to monotherapy with infliximab. *Clin Rheumatol.* 2007;26:2001–3.

253. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT, Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. *Circulation.* 2003;107:3133–40.

254. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2013;62:e147–239.

255. Echt DS, Liebson PR, Mitchell LB, Peters RW, Obias-Manno D, Barker AH, et al. Mortality and morbidity in patients receiving encainide, flecainide, or placebo. The Cardiac Arrhythmia Suppression Trial. *N Engl J Med.* 1991;324:781–8.

256. Epstein AE, Dimarco JP, Ellenbogen KA, Estes NAM, Freedman RA, Gettes LS, et al. 2012 ACCF/AHA/HRS focused update incorporated into the ACCF/AHA/HRS 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. *J Am Coll Cardiol.* 2013;61:e6–75.

257. Brignole M, Auricchio A, Baron-Esquivias G, Bordachar P, Boriani G, Breithardt O-A, et al. 2013 ESC Guidelines on cardiac pacing and cardiac resynchronization therapy: the Task Force on cardiac pacing and resynchronization therapy of the European Society of Cardiology (ESC). Developed in collaboration with the European Heart Rhythm Association (EHRA). *Eur Heart J.* 2013;34:2281–329.

258. Epstein AE, Dimarco JP, Ellenbogen KA, Estes NAM, Freedman RA, Gettes LS, et al. ACC/AHA/HRS 2008 guidelines for Device-Based Therapy of Cardiac Rhythm Abnormalities: executive summary. *Heart Rhythm.* 2008;6:934–55.

259. Zaidi AR, Zaidi A, Vaitkus PT. Outcome of heart transplantation in patients with sarcoid cardiomyopathy. *J Heart Lung Transplant.* 2007;26:714–7.

260. Khan R, Tweedie EJ, Pflugfelder PW, White JA. Cardiac sarcoid in a heart transplant recipient: detection with cardiac magnetic resonance imaging.

Transplant Proc. 2010;42:1976–8.

261. Yager JEE, Hernandez AF, Steenbergen C, Persing B, Russell SD, Milano C, et al. Recurrence of cardiac sarcoidosis in a heart transplant recipient. *J Heart Lung Transplant.* 2005;24:1988–90.

262. Burke WM, Keogh A, Maloney PJ, Delprado W, Bryant DH, Spratt P. Transmission of sarcoidosis via cardiac transplantation. *The Lancet.* 1990;336:1579.

263. Frustaci A, Chimenti C, Pieroni M, Gentiloni N. Giant cell myocarditis responding to immunosuppressive therapy. *Chest.* 2000;117:905–7.

264. Desjardins V, Pelletier G, Leung TK, Waters D. Successful treatment of severe heart failure caused by idiopathic giant cell myocarditis. *Can J Cardiol.* 1992;8:788–92.

265. Menghini VV, Savcenko V, Olson LJ, Tazelaar HD, Dec GW, Kao A, et al. Combined immunosuppression for the treatment of idiopathic giant cell myocarditis. *Mayo Clin Proc.* 1999;74:1221–6.

266. Chaudhry MA, Correa A, Lee C, Yoon A, Grazette L, Saremi F, et al. Modern day management of giant cell myocarditis. *Int J Cardiol.* 2015;178:82–4.

267. Nieminen MS, Salminen US, Taskinen E, Heikkilä P, Partanen J. Treatment of serious heart failure by transplantation in giant cell myocarditis diagnosed by endomyocardial biopsy. *J Heart Lung Transplant.* 1994;13:543–5.

268. Brilakis ES, Olson LJ, Berry GJ, Daly RC, Loisance D, Zucker M, et al. Survival outcomes of patients with giant cell myocarditis bridged by ventricular assist devices. *ASAIO J.* 2000;46:569–72.

269. Iwai K, Tachibana T, Takemura T, Matsui Y, Kitaichi M, Kawabata Y. Pathological studies on sarcoidosis autopsy. I. Epidemiological features of 320 cases in Japan. *Acta Pathol Jpn.* 1993;43:372–6.

270. Takaya Y, Kusano KF, Nakamura K, Ito H. Comparison of Outcomes in Patients With Probable Versus Definite Cardiac Sarcoidosis. *Am J Cardiol*. 2015;115:505-9.

271. Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. *Autoimmun Rev.* 2015;14:174–80.

272. Blankstein R, Osborne MT, Waller AH, Murthy VL, Dorbala S, Stevenson WG, et al. Reply: (18)F-FDG imaging in patients with "suspected," but not “proven,” sarcoidosis. *J Am Coll Cardiol*. 2014;64:631.

273. Ramos-Casals M, Brito-Zerón P, Kostov B, Sisó-Almirall A, Bosch X, Buss D, et al. Google-driven search for big data in autoimmune geoepidemiology: analysis of 394,827 patients with systemic autoimmune diseases. *Autoimmun Rev.* 2015;14:670–9.

274. Nery PB, Beanlands RS, Nair GM, Green M, Yang J, McArdle BA, et al. Atrioventricular block as the initial manifestation of cardiac sarcoidosis in middle-aged adults. *J Cardiovasc Electrophysiol.* 2014;25:875–81.

275. Fairweather D, Cooper LT, Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy. *Curr Probl Cardiol*. 2013;38:7–46.

276. Baughman RP, Grutters JC. New treatment strategies for pulmonary sarcoidosis: antimetabolites, biological drugs, and other treatment approaches. *Lancet Respir Med*. 2015;3:813–22.

277. Rybicki BA, Hirst K, Iyengar SK, Barnard JG, Judson MA, Rose CS, et al. A sarcoidosis genetic linkage consortium: the sarcoidosis genetic analysis (SAGA) study. *Sarcoidosis Vasc Diffuse Lung Dis.* 2005;22:115–22.

278. Cooper LT, Blauwet LA. When should high-grade heart block trigger a search for a treatable cardiomyopathy? *Circ Arrhythm Electrophysiol*. 2011;4:260–1.

279. Simonen P, Lehtonen J, Kandolin R, Schildt J, Marjasuo S, Miettinen H, et al. F-18-Fluorodeoxyglucose Positron Emission Tomography-Guided Sampling of Mediastinal Lymph Nodes in the Diagnosis of Cardiac Sarcoidosis. Am J Cardiol. 2015;116:1581-5.

280. Authors/Task Force Members, Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC)Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). *Eur Heart J.* 2015 Aug 29:ehv316–87.

281. Gonzalez JA, Kramer CM. Role of Imaging Techniques for Diagnosis, Prognosis and Management of Heart Failure Patients: Cardiac Magnetic Resonance. *Curr Heart Fail Rep.* 2015;12:276–83.

282. Sarikaya I. Cardiac applications of PET. *Nucl Med Commun.* 2015;36:971-85.

Recent Publications in this Series

85/2015 And Demir
Clinical Use of Urinary Gonadotropin Determinations in Children and Adolescents
86/2015 Raisa Haavikko
Synthesis of Betulin Derivatives with New Bioactivities
87/2015 Hongxin Chen
Heterobasidion annosum sensu stricto Pathogenesis: Bioinformatic and Functional Study of Cerato-Platanin Family Proteins
88/2015 Kristiina Rajamäki
Role of the Inflammasome Pathway in Atherosclerosis
89/2015 Pirkko Kriikku
Toxicological Abuse Profile of New Recreational Drugs in Driving-Under-The-Influence and Post-Mortem Cases in Finland
90/2015 Eeva Mustonen
Red Clover Isoflavonoids in Feed, Plasma and Milk of Ruminants
91/2015 Flavia Restitutti
Central, Cardiovascular and Metabolic Effects of Dexmedetomidine Associated with the Selective Peripheral Alpha2-adrenoceptor Antagonist MK-467 in Dogs
92/2015 Katariina Nurmi
Prevention of Inflammatory Cellular Responses by Ethanol and Hemin – Interplay Between Inflammasomes and Processes Inhibiting Inflammation
93/2015 Liang He
Rare Genetic Variants and Complex Diseases - a Bayesian Approach
94/2015 Jani Saksi
Stroke-Associated Carotid Atherosclerosis - a Role for FABP4 in Atheroma Vulnerability and Cardiometabolic Risk
95/2015 Otto Hemminki
Cancer Immunotherapy with a Gene Modified Serotype 3 Oncolytic Adenovirus
96/2015 Jukka Koskela
Individual Trajectories in Asthma and COPD: A Longitudinal Perspective to Obstructive Lung Disease
97/2015 Miira Klemetti
Trends In Obstetric and Perinatal Outcomes of Women with Type 1 Diabetes During 1988-2011 – A Finnish Population-Based Observational Study
98/2015 Ileana B. Quintero
Physiological Functions of Prostatic Acid Phosphatase
99/2015 Minna Matikainen
Launching New Products in the Finnish Pharmaceutical Industry: A Relationship Approach
100/2015 Päivi Ylä-Anttila
Phagophore Membrane Connections and RAB24 in Autophagy
101/2015 Kaisa Kyöstilä
Identification of Novel Mutations and Molecular Pathways for Canine Neurodegeneration and Chondrodysplasia
102/2015 Emmi Joensuu
Epigenetic Alterations in Sporadic and Familial Cancers
103/2015 Elina Reponen
Preoperative Risk-Assessment Methods, Short-Term Outcome, and Patient Satisfaction in Elective Cranial Neurosurgery

Helsinki 2015 ISSN 2342-3161 ISBN 978-951-51-1768-7